TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

CONVERGENCE OF RAS AND PP2A ACTIVITIES ON CHROMATIN REPRESSOR COMPLEXES

Mukund Sharma

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1778 | MEDICA – ODONTOLOGICA | TURKU 2024

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

CONVERGENCE OF RAS AND PP2A ACTIVITIES ON CHROMATIN REPRESSOR COMPLEXES

Mukund Sharma

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1778 | MEDICA – ODONTOLOGICA | TURKU 2024

University of Turku

Faculty of Medicine
Institute of Biomedicine
Pathology
Turku Doctoral Program of Molecular Medicine
Turku Bioscience Centre

Supervised by

Professor Jukka Westermarck, M.D., Ph.D.
Institute of Biomedicine
Turku Bioscience Centre
University of Turku
Turku Finland

Reviewed by

Associate Professor Chiara Ambrogio
Molecular Biotechnology Center (MBC)
University of Torino
Torino, Italy

Associate Professor Sakari Vanharanta
Translational Cancer Medicine Program
University of Helsinki
Helsinki, Finland

Opponent

Professor Dr. Tilman Brummer
Institute of Molecular Medicine and Cell Research
University of Freiburg
Freiburg, Germany

The originality of this publication has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service.

ISBN 978-951-29-9624-7 (PRINT)
ISBN 978-951-29-9625-4 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)
Painosalama, Turku, Finland 2024

To My Family

UNIVERSITY OF TURKU
Faculty of Medicine
Institute of Biomedicine
Pathology
MUKUND SHARMA: Convergence of RAS and PP2A activities on chromatin repressor complexes
Doctoral Dissertation, 186 pp.
Turku Doctoral Programme of Molecular Medicine
Turku Bioscience
January 2024

ABSTRACT

Protein Phosphatase 2A (PP2A) is a serine-threonine phosphatase and, through its de-phosphorylation activity, mostly downregulates signaling pathways, while RAS belongs to the family of small GTPase and activates various kinases resulting in the activation of cellular signaling. Decades back, it was shown that the inhibition of PP2A is a prerequisite for human cell transformation by the oncogenic RAS. Despite the mounting evidence highlighting RAS and PP2A-mediated protein phosphorylation to be of paramount significance in cancer biology, none of the studies have systematically addressed the cooperation between RAS and PP2A in regulating global protein phosphorylation.

My thesis focuses on the cooperation between the tumor suppressor PP2A and an oncogene RAS in regulating protein phosphorylation in cancer cells. By integrating the RAS- and PP2A-regulated phosphoproteomics data we found that the cellular proteome, is specifically enriched for RAS- and PP2A-regulated phosphosites on proteins involved in regulating various epigenetic modifications such as DNA methylation, histone methylation/acetylation and chromatin remodeling.

In the present work, I have validated the impact of the PP2A- and RAS-regulated phosphosites in the functional regulation of epigenetic proteins. Using multi-omics analysis, this study has further uncovered the opposing roles of RAS and PP2A in regulating gene expression. The present study has also compared the two nuclear inhibitors of PP2A, PME-1, and SET, and discovered the diversity of their roles in oncogenic transcription and DNA methylation. Collectively, this research work indicates that in cancer, RAS hyperactivity and PP2A inhibition converge on phosphorylation of epigenetic proteins and has a vital role in oncogenic transcription.

KEYWORDS: Lung cancer, Phosphorylation, PP2A, RAS, PME-1, SET, transcription, Epigenetics, DNMT1, HDAC, methylation, acetylation, chromatin remodeling

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Biolääketieteen laitos
Patologia
MUKUND SHARMA: RAS onkoproteiinin ja PP2A kasvunestäjän yhteisvaikutus geeninluentaan syövässä
Väitöskirja, 186 s.
Molekyylilääketieteen tohtoriohjelma
Turun biotiedekeskus
Tammikuu 2024

TIIVISTELMÄ

Proteiinifosfataasi 2A (PP2A) on seriini-treoniinifosfataasi, joka vaimentaa monien syövän kannalta tärkeiden signalointireittien aktiivisuutta, kun taas RAS, joka kuuluu pienien GTPaasi proteiinien perheeseen, aktivoi solusignalointikinaaseja syövässä. Samanaikainen RAS:in aktivaatio ja PP2A:n inhibitio on edellytyksenä ihmisen solujen muuttumiselle pahanlaatuisiksi. Kuitenkaan tähän mennessä ei ole systemaattisesti tutkittu RAS:in ja PP2A:n yhteistyötä proteiinien fosforylaation säätelyssä syöpäsoluissa. Tämä onkin ollut väitöskirjatutkimukseni keskeinen kysymys. Yhdistämällä proteomiikka-analyysit RAS- ja PP2A-muokatuista syöpäsoluista havaitsimme, että RAS:in ja PP2A:n säätelemät fosforylaatiokohdat esiintyvät rikastuneina proteiineissa, jotka osallistuvat erilaisten epigeneettisten modifikaatioiden kuten DNA:n metylaation, histonien metylaation/asetylaation sekä kromatiinin uudelleenmuokkauksen säätelyyn. Osoitan tässä työssä, että nämä fosforylaatiokohdat vaikuttavat epigeneettisten proteiinien toiminnan säätelyyn. Tutkimuksessa havaittiin myös, että RAS:lla ja PP2A:lla on erilainen rooli geenien ilmentymisen säätelyssä. Havaitsin myös, että kahdella PP2A:ta estävällä tumaproteiinilla, PME-1 ja SET, on erilainen rooli syöpään liittyvien geenien luennassa sekä DNA:n metylaation säätelyssä. Kaiken kaikkiaan väitöskirjatutkimukseni osoittaa, että RAS:in yliaktiivisuuden ja PP2A:n eston vaikutukset kohtaavat epigeneettisten proteiinien fosforylaation säätelyssä ja että tällä on tärkeä rooli onkogeenisessä geeninluennassa.

AVAINSANAT: Keuhkosyöpä, fosforylaatio, PP2A, RAS, PME-1, SET, transkriptio, epigenetiikka, DNMT1, HDAC, metylaatio, asetylaatio, kromatiinin uudelleenmuokkaus

Table of Contents

Abbreviations

AdenoCA	Adenocarcinoma
AKT	Protein kinase B
ALK	Anaplastic lymphoma kinase
AZA	Azacitidine
CHD3	Chromodomain Helicase DNA Binding Protein 3
CIP2A	Cancerous inhibitor of PP2A
CK2α	Casein kinase 2, alpha subunit
DAC	Decitabine
DNMT1	DNA methyltransferase 1
E2F1	Transcription factor E2F1
EGFR	Epidermal growth factor receptor
ERK	Extracellular signal-regulated kinase
GAP	GTPase activating protein
GEF	Guanine nucleotide exchange factors
HBEC	Human bronchial epithelial cells
HDAC1	Histone deacetylase 1
HDAC2	Histone deacetylase 1
HEK	Human embryogenic kidney cells
HER4	Human epidermal growth factor receptor 4
hTERT	human telomerase reverse transcriptase enzyme
KDa	Kilo dalton
KDM1A	Lysine-specific demethylase 1A
KMT2A	histone methyltransferase 2A
LCLC	Large cell carcinoma
LT	Simian virus large T antigen
MAPK	Mitogen-activated protein kinase
MET	Mesenchymal–epithelial transition tyrosine kinase receptor
MGMT	O-6-methylguanine-DNA methyltransferase
mTOR	Mammalian target of rapamycin
myc	C-MYC proto-oncogene
NSCLC	Non-small cell lung cancer

NTRK	Neurotrophic receptor tyrosine kinase
NuRD	Nucleosome Remodeling and Deacetylase complex
PI3K	Phosphatidylinositol 3-kinase, catalytic subunit alpha
PLSCR4	Phospholipid Scramblase 4
PME-1	Protein phosphatase methyl esterase 1
PP2A	Protein phosphatase 2A
PRMT	Arginine N-methyltransferase
PTEN	Phosphatase and tensin homolog
RAF	RAF serine/threonine-protein kinases
RAS	RAS-family small GTPases
SAH	S-adenosylhomocysteine
SAM	S-adenosylmethionine
SCLC	Small cell lung cancer
SET	SET nuclear proto-oncogene
SQLC	Squamous cell carcinoma
ST	Simian virus small T antigen
SV40	Simian vacuolating virus 40
TET	Ten eleven translocase
TGFB2	Transforming growth factor-beta 2
TP53	Tumor protein p53

List of Original Publications

This dissertation by Mukund Sharma is based on the following original publications, which are referred to in the text by their Roman numerals:

I Anna Aakula*, **Mukund Sharma***, Francesco Tabaro, Reetta Nätkin, Jesse Kamila, Henrik Honkanen, Matthieu Schapira, Cheryl Arrowsmith, Matti Nykter, and Jukka Westermarck. RAS and PP2A activities converge on epigenetic gene regulation. Life science alliance 6, no. 5 (2023).

II **Mukund Sharma**, Francesco Tabaro, Jesse Kamila, Matti Nykter, and Jukka Westermarck. Transcriptional readouts of nuclear PP2A inhibitor proteins PME-1 and SET reveal differential biological outcomes. (Manuscript).

*Equal contributions

1 Introduction

Hyperactivation of phosphorylation-dependent signaling pathways due to mutations in RAS proteins is a hallmark of various human cancers (Hanahan, 2022). Multiple strategies have been used to target RAS-driven cancers, but most have failed due to the activation of alternate pathways downstream of RAS, or the development of drug resistance, making it essential to find new targets involved in RAS signaling (A. R. Moore et al., 2020). Each of the PP2A subunits can be coded by multiple genes, resulting in various isoforms. These isoforms can further assemble in various combinations, resulting in a wide range of the PP2A holoenzyme complexes, empowering PP2A to catalyse a wide range of substrates as well as defining its substrate specificity. Protein Phosphatase 2A is a tumor suppressor, and the oncogenic RAS-mediated human cell transformation requires the simultaneous inhibition of PP2A, indicating PP2A as a major antagonist to RAS activity (Hahn et al., 1999, 2002; Rangarajan et al., 2004). Various studies have demonstrated that PP2A inhibition by oncoproteins CIP2A, PME1, and SET facilitates RAS-mediated malignant cell transformation (Junttila et al., 2007; Kauko et al., 2015, 2018; Westermarck, 2018). Overexpression of PP2A inhibitory proteins is commonly observed in cancer cells (Kauko & Westermarck, 2018). Recent advances in the therapeutic reactivation of PP2A using small molecular activators (SMAP) have opened a new window of opportunity in targeting RAS-driven cancers. SMAP treatment was effective in thwarting the growth of mutant KRAS-driven mouse xenografts and transgenic models. When used in combination with MEK inhibitors, SMAP synergizes in inducing regression of KRAS-driven mouse models (Farrington et al., 2020; Kauko et al., 2018; Merisaari et al., 2020; Sangodkar et al., 2017). These developments further cement the role of PP2A in thwarting RAS-driven tumor growth. However, the convergence of their activities in regulating global phosphorylation events has never been addressed.

To systematically characterize the target mechanisms co-regulated by PP2A and RAS synergy, our group had previously used phosphoproteomics mass spectrometry to compare phosphoproteome profiles in cancer cells upon RAS inhibition and PP2A modulation (Kauko et al., 2015, 2020a). Further integrative analysis of the data showed that RAS and PP2A regulating highly similar functions, and their activities

converge in regulating similar phosphorylation sites on proteins involved in epigenetic pathways. This suggests that the phosphoregulation of epigenetic complexes by RAS and PP2A might be a relevant mechanism for human cell transformation and oncogenesis. This Ph.D. thesis is founded on this discovery, and in the present study, I have focused on epigenetic complexes that have been previously shown to be important for RAS-mediated functions, but there is no prior knowledge about the role of PP2A-mediated dephosphorylation in their regulation. This thesis aims to highlight the functional contribution of RAS & PP2A-regulated phosphorylation events in epigenetic gene regulation and RAS-driven oncogenesis.

Despite decades of research, RAS-driven cancers are still untreatable. It is essential to devise alternate strategies in the fight against RAS-driven cancers. My research will help in understanding how RAS and PP2A synergize and regulate epigenetic complexes. Phosphatases as a treatment strategy have long been ignored. With the discovery of small molecule activators of PP2A, it is now possible to add a new dimension to targeting cancer. Combinatorial therapy by PP2A activation followed by oncoprotein inhibition should increase efficacy and treatment response. Various epigenetic proteins aid in tumor growth and progression, and drugs targeting them are in different phases of clinical trials. Recent work from our group showed that PP2A inhibition results in resistance against HDAC and Bromodomain inhibitors (Kauko et al., 2020a). PP2A is inhibited by multiple means in most cancers. Re-activation of PP2A using small molecules in combination with inhibitors against epigenetic complexes can be a robust strategy to tackle RAS-driven cancers.

In my Ph.D. work, I have systematically characterized the opposing roles of RAS and PP2A in regulating various epigenetic mechanisms. For instance, I found that PP2A and RAS activities oppose the chromatin recruitment of histone deacetylase 1 and 2. This study found that PP2A and RAS oppose various epigenetic processes such as chromatin recruitment of histone deacetylase 1 and 2, the interaction between RNF168-TP53B1 mediated through a co-regulated phosphorylation site on RN168, stability of CHD3, and overall gene expression programming. This study further identified PP2A as a facilitator of epigenetic mechanisms such as DNA methylation and chromatin condensation, which are involved in gene repression. In this thesis, I have also addressed the role of the two nuclear inhibitors of PP2A (PME-1 and SET) and compared their transcriptomes and methylomes. The role of PME-1 in regulating DNA methylation by downregulating the methyltransferase DNMT1 is another highlight of this study.

My thesis work will help in elucidating molecular mechanisms behind RAS and PP2A synergy in the regulation of epigenetic complexes, and how their dysregulation leads to cancer. As proof of principle, I have shown that the combinatorial therapy by PP2A activation and targeting HDAC synergize and could be used as an alternate strategy to treat RAS-driven cancers. Moreover, we have

uncovered a valuable database of phosphorylation sites co-regulated by PP2A and RAS activity, which needs further investigation for a better understanding of the cancer biology regulated by protein phosphorylation.

2 Review of the Literature

2.1 Lung Cancer

With a projected annual incidence of two million new diagnoses and 1.76 million fatalities, lung cancer is among the most often diagnosed malignancies and accounts for around 20% of cancer-related deaths globally (Thai et al., 2021). Lung cancer presents various symptoms and indications based on its anatomic location because it is a very diverse disease that may develop at several places along the bronchial tree. Most cases of lung cancer are diagnosed when the disease has already progressed to the advanced stage (Lemjabbar-Alaoui et al., 2015).

The two primary subtypes of lung cancer include non-small cell lung cancer (NSCLC) accounting for 85% of all the cases and small cell lung cancer (SCLC) accounting for 15% of all lung cancer cases. Non-small include adenocarcinoma (AdenoCA), squamous cell carcinoma (SQCLC), and large cell carcinoma (LCLC). About 25%–30% of all lung malignancies are squamous cell lung cancers (SQCLC), which often start in the primary bronchi and spread to the carina. Adenocarcinomas (AdenoCA) develop in peripheral bronchi and account for around 40% of all cases of lung cancer. Large cell carcinoma (LCLC) represents about 10% of all lung cancers,grows in the peripheral regions of the lung, and shows aggressive growth. Small cell lung cancers (SCLC) are of neuroendocrine origin and are mostly associated with the smoking history of the patients. SCLC is highly aggressive and comprises 10% of all lung cancer cases (Lemjabbar-Alaoui et al., 2015; Rudin et al., 2021; Thai et al., 2021).

The transformation of a normal cell into a tumor cell is initialized through a series of alterations in the genetic and epigenetic landscape, which further accumulate through the process of clonal expansion, resulting in cancer development. Early detection and characterization of the changes in the cellular microenvironment can significantly help in the prevention and treatment of the disease. Understanding a patient's tumor features and genetics will significantly improve their prognosis and treatment choice (Lemjabbar-Alaoui et al., 2015). In the past century, the main course of cancer treatment included the use of chemotherapy based on the patient’s tumor histology. In contrast, the emergence of predictive biomarkers in the last two decades has opened up novel possibilities for cancer treatment guided by targeted

therapy. Tumor histology continues to be used as a predictor of response in chemotherapy. For instance, the non-squamous NSCLC patients benefited from pemetrexed treatment, but squamous NSCLC patients showed no change in overall survival between treatment and placebo groups (Standfield et al., 2011; Šutić et al., 2021). The implementation of cancer therapy for the treatment of lung cancer is significantly influenced by the genetic changes in cancer cells, making the identification of these cancer-specific biomarkers a prerequisite for the successful treatment of lung cancer. Lung cancer shows alterations in the cellular signaling pathways that regulate various biological processes.

RAS proteins show one of the highest mutation rates in human cancers, and KRAS-driven NSCLC accounts for a quarter of all NSCLC cases. The highest frequency of KRAS mutations detected in lung cancer is the G12C mutation, resulting in a constitutively active form of the KRAS protein. Various efforts have been made in direct and indirect targeting of the RAS pathway, and their failure is attributed to the development of resistance and activation of alternate signaling pathways or toxicity (J. Luo et al., 2022). Direct targeting of the mutant RAS was first achieved by the development of a small molecule that bound to the mutant cysteine at position 12 of the KRAS protein, locking it into a GDP-bound inactive state (Ostrem et al., 2013). Based on this finding, the irreversible covalent inhibitors sotorasib (AMG 510) and adagrasib (MRTX849) against the G12C mutant KRAS were developed, which showed complete tumor regression in mouse models by inhibiting the MAPK signaling (Canon et al., 2019; Fell et al., 2020). Both drugs have been approved for treating KRAS G12C mutant NSCLC (Nakajima et al., 2022; Dhillon, 2023).

Mutations in the EGFR receptor lead to hyperactivation of various signaling pathways in cancer and are pivotal in predicting the response to EGFR tyrosine kinase inhibitors (TKI). Around 85% of the EGFR mutations comprise the exon 19 deletion and exon 21 (L858R) mutations and are sensitive to the EGFR inhibitors. In contrast, the insertion at exon 20 of the EGFR receptor drives resistance against the inhibitors. The Food and Drug Administration (FDA) of the United States has at present authorized five TKIs for treating NSCLC, which have significantly improved disease-free and progression-free survival in patients. Erlotinib and gefitinib are reversible tyrosine kinase inhibitors of the first generation while afatinib and dacomitinib belong to the second generation and bind irreversibly to the EGFR receptor. The EGFR T790M mutation leads to drug resistance against the first-generation TKI, and to treat cancers harboring this mutation the third generation of TKI osimertinib is employed (Lemjabbar-Alaoui et al., 2015; Šutić et al., 2021).

Mutations in BRAF protein occur in around 4% of NSCLC cases. Most BRAF-positive NSCLC patients have a V600E mutation, resulting in constitutively active

BRAF, which then phosphorylates the downstream effector MEK, leading to hyperactivated MAPK signaling. NSCLC patients harboring the V600E mutation are treated using combination therapy using the BRAF inhibitor dabrafenib and the MEK inhibitor trametinib (Planchard et al., 2017). The mesenchymal–epithelial transition tyrosine kinase receptor (MET) is involved in activating various signaling pathways, including MAPK/ERK and PI3K/AKT. MET alterations are detected in 3 to 5% of NSCLC of lung cancer and include amplification of the MET gene and mutations in its kinase domain and exon 14 skipping mutations (Friedlaender et al., 2020). NSCLC with MET exon 14 skipping mutations are treated using the MET inhibitors capmatinib and tepotinib (Thai et al., 2021).

Various oncogenic signaling pathways, such as RAS and PI3K/AKT, are activated by the anaplastic lymphoma kinase (ALK) receptor. Alterations in the gene coding for the ALK are found in 3–5% of NSCLC patients. This generates an oncogenic ALK fusion protein that hyperactivates the downstream signaling module. Crizotinib was the first drug to get approval for treating ALK rearrangements, and it achieved a highly significant increase in median overall survival compared to chemotherapy as a first-line therapy (B. J. Solomon et al., 2018). However secondary mutations such as L1196M, C1156Y, and L1152R drive acquired drug resistance to crizotinib (Choi et al., 2010; Sasaki et al., 2011). The second generation of ALK TKIs, such as alectinib, brigatinib, and ensartinib, overcome crizotinib resistance and improve progression-free survival in NSCLC. Acquired mutations in the ALK gene that drive resistance to the second-generation ALK inhibitor alectinib have been identified (Katayama et al., 2014). Lorlatinib is a new third-generation ALK TKI that can cross the blood-brain barrier and has shown more effectiveness against ALK-resistant mutations and improved progression-free survival than second-generation ALK TKIs (B. Solomon et al., 2020).

The rearrangement in ROS1 tyrosine kinase receptor occurs in 1–3% of adenocarcinomas, and similar to ALK, they primarily occur in younger populations without a history of cigarette smoking. The first-line ALK TKI crizotinib is also authorized for NSCLC with ROS rearrangement (Šutić et al., 2021). Around 1% of NSCLC patients harbor NTRK (Neurotrophic receptor tyrosine kinase) gene mutations that giverise to the oncogenic fusion proteins. Larotrectinib and entrectinib, are the two TRK inhibitors that the FDA has given fast-track approval for the treatment of solid tumors that show NTRK alterations (Thai et al., 2021). Another oncogenic fusion that occurs in 1–2% of lung adenocarcinomas is the RET (Rearranged During Transfection) fusion, leading to the dimerization and activation of RET kinases (Šutić et al., 2021).

SCLC shows a very high mutation rate. However, compared with NSCLC, genomic profiling of SCLC has not successfully identified the subtypes driven by specific mutations (Semenova et al., 2015). SCLC is characterized by mutations

leading to the loss of function of the tumor suppressor genes TP53 and retinoblastoma (RB) in 75 to 90% of the cases. Other commonly occurring mutated genes include PTEN and NOTCH (Rudin et al., 2021). Amplification of the MYC family genes occurs in 20–30% of SCLC tumors. In addition to MYC, the amplification of the gene coding for fibroblast growth factor receptor 1 (FGFR1) and GNAS, coding for the α-subunit of the G protein is frequently found to be amplified. Around 8% of SCLC tumors show the inactivation of the histone methyltransferase (KMT2D) gene (Rudin et al., 2021).

2.2 Epigenetic regulation of gene expression

A single copy of the human genome comprises three billion base pairs which correspond to a length of around two meters. This large amount of genetic material is packed inside the nucleus with an average diameter of 10 μM. DNA is tightly wrapped around the histone proteins to facilitate the compaction of the entire genetic material in the nucleus. These polymeric complexes of histones and DNA are known as chromatin (Ar & Jj, 2015).

Chromatin comprises repeating structures called nucleosomes consisting of a nucleosome core, a linker DNA, and a linker histone. The nucleosome core is formed by wrapping 147 base pairs of DNA around the octameric complex formed by two copies of each of the four histone proteins (H2A, H2B, H3, H4). The adjacent nucleosome cores are connected by linker DNA bound to the linker histones (H1 or H5), giving rise to fundamental units called the chromatosome (McGinty & Tan, 2015; Simpson, 1978). The part of the core histones (25-30%) devoid of the wrapped DNA constitutes the histone tail region. The tail region of histones is evolutionarily conserved and undergoes various post translational modifications. These epigenetic modifications on the histone tail regulate chromatin organization and accessibility of the nucleosomal DNA to multiple transcription factors and DNA binding proteins (Ar & Jj, 2015; D. Y. Lee et al., 1993; Z. Yang et al., 2005).

The term Epigenetics comprises the heritable changes on the genome involved in regulating the gene expression without causing a change in the underlying DNA sequence. These modifications are passed on to the offspring (heritable) and play a very important role in regulating thegene expression and development (Deichmann, 2016). Various epigenetic modifications that occur on the chromatin include histone modifications, DNA methylation, and chromatin remodeling. This involves the addition or removal of chemical tags on the genome and employs a group of specialized proteins that work in tandem to regulate various biological processes. These proteins can either add a chemical modification (writers) ,remove an existing modification (erasers) or interpret these modifications (readers) and further proceed to bring the desired changes (Audia & Campbell, 2016).

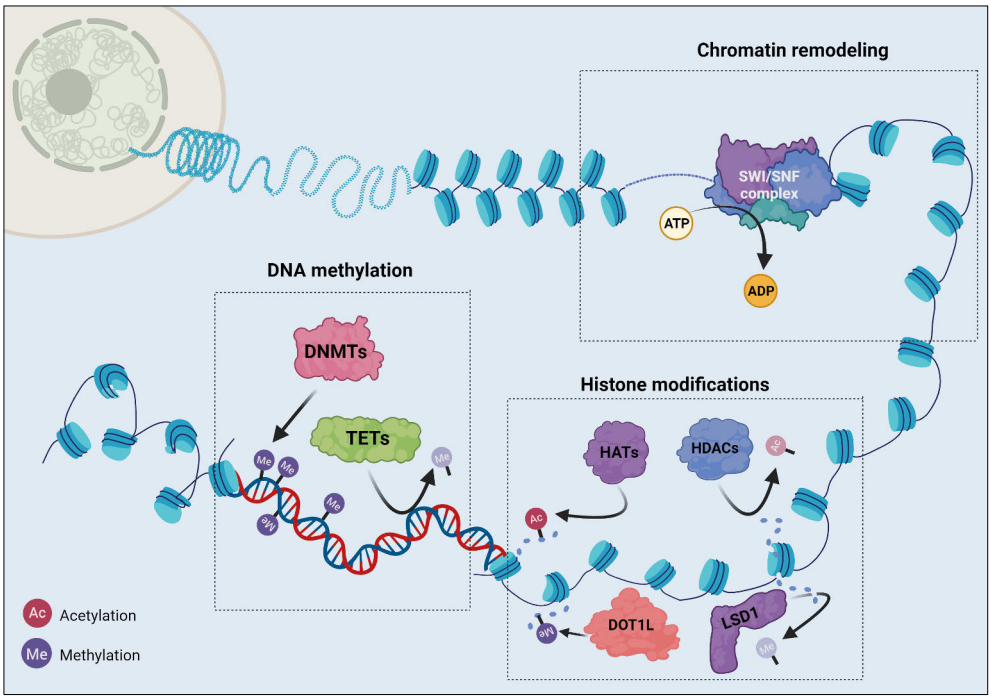

Figure 1. Epigenetic mechanisms involved in gene regulation. Created with BioRender.com.

2.2.1 DNA methylation

DNA methylation is a major epigenetic regulatory mechanism and involves the chemical modification of DNA by the addition of methyl groups. DNA methylation was first discovered in mammals in 1948 by Rollin Hotchkiss while separating the purines and pyrimidines using paper chromatography. Rollin noted that the chromatogram of the DNA preparation from the calf thymus had an additional peak for cytosine resides and called it epicytosine (Hotchkiss, 1948). The role of DNA methylation in regulating gene expression was reported in later studies which observed specific genes to be differentially methylated across various cell types (Mandel & Chambon, 1979). Further, the methylation of genes is inversely correlated with its expression, indicating DNA methylation as a chemical modification that suppresses gene expression (Jd & Gd, 1979; M. T. Kuo et al., 1979). The distribution of DNA methylation patterns across the genome regulates various biological functions such as gene expression, genomic imprinting, and inactivation of the X-chromosome (L. D. Moore et al., 2013).

The primary site of DNA methylation in vertebrates is the cytosine residue adjacent to a guanine residue (CpG). The CpG sites are found throughout the human genome including the intergenic regions and gene bodies and their methylation is mainly

associated with gene inactivation. The CpG sites are primarily methylated across the human genome except for short stretches of CpG repeats called CpG islands. The CpG islands were initially discovered in mouse sperm as stretches of unmethylated DNA and were later called "methylation-free zones" (Bird et al., 1985; Gardiner-Garden & Frommer, 1987). They are denoted by stretches of up to 1000 bp long DNA with a GC composition of more than 60%. Promoters of most of the housekeeping genes are found to contain CpG islands, indicating them as sites for transcriptional initiation (Deaton & Bird, 2011). Methylation at CpG islands results in gene silencing and plays an important role in various processes, such as establishing gene imprinting and regulating cell differentiation (Wutz et al., 1997; Meissner et al., 2008).

The enzymes that catalyse the methylation of DNA (writers) are known as DNA methyltransferases (DNMT). The five methyltransferases coded by the human genome are DNMT1, DNMT3a and DNMT3b, and DNMT3L. The first mammalian DNA methyltransferase to be cloned and sequenced was DNA methyltransferase 1 (Dnmt1) (Bestor et al., 1988). DNMT1 is also called maintenance methyltransferase as it copies the existing methylation patterns to the newly synthesized DNA. DNMT3a and 3b are known as native or de novo DNA methyltransferases since they establish the methylation patterns during early development. De novo methylation is important in tissue differentiation by establishing tissue-specific gene patterns. Knockout of both these methyltransferases was shown to impair the de novo methylation in embryonic stem cells and embryos while not affecting the maintenance of the methylation imprinting of the bulk DNA (Okano et al., 1999). DNMT3L has no enzymatic activity but enhances the DNA methylation rate by forming a complex with DNMT3a or 3b (Bourc'his et al., 2001).

The DNA methylation reaction involves transferring a methyl group to carbon 5 of the cytosine residue. SAM (S-adenosylmethionine) is a universal methyl donor for all methyltransferase reactions and is reduced to SAH (S-adenosylhomocysteine) upon completion of the reaction (Figure 2). DNMT rotates the target cytosine base in its catalytic pocket and transfers the methyl group from SAM to the carbon five of the cytosine. DNMT1 shows a preference for longer substrates and is a processive enzyme that adds a methyl group to long stretches of the DNA. DNMT1 binds to the DNA and, upon recognition of its substrate, undergoes a conformational change leading to its activation. The enzyme then adds the methyl group to its target and continues searching for other sites without exchanging the DNA strands. DNMT1 mainly catalyses the methylation of hemimethylated substrates, which possess methylated CpG on only one of the strands (Hermann et al., 2004). DNMT3B methylates DNA in a processive manner like DNMT1, while DNMT3A acts in a distributive manner (Gowher & Jeltsch, 2002). DNMT3A adds methyl groups to cytosines and then falls off its substrate DNA, leading to methylated, partially methylated, and unmethylated DNA populations.

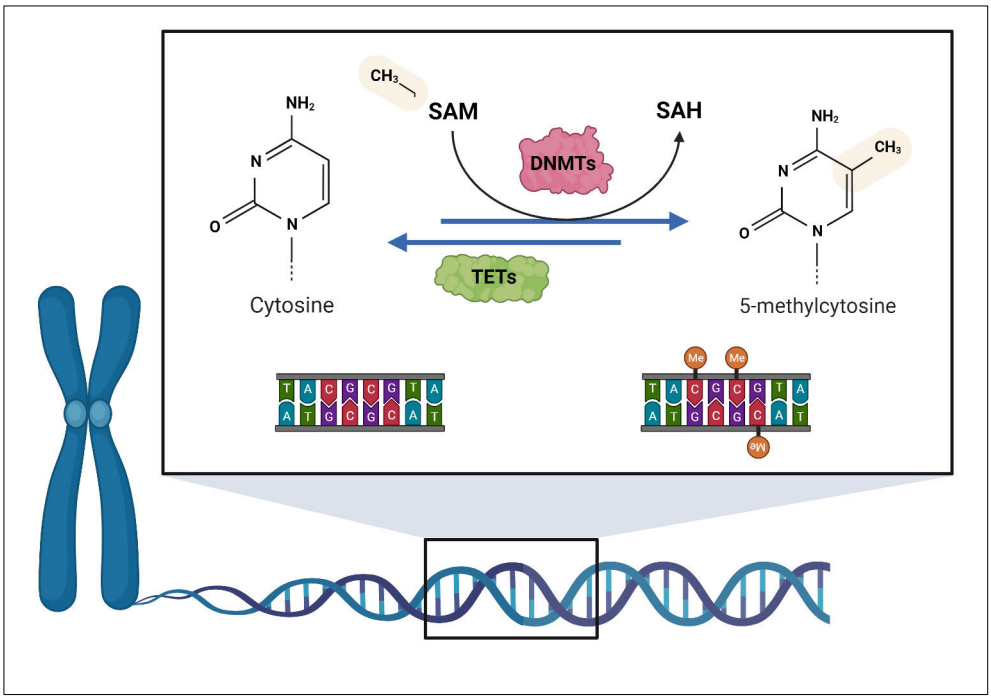

Figure 2. Mechanism of DNA methylation. DNMT transfers a methyl group from the methyl donor SAM to the cytosine residue. SAM (S-adenosylmethionine) is reduced to SAH (S-adenosylhomocysteine) in this process. DNA is demethylated by the activity of TET enzymes. Created with BioRender.com.

DNA demethylation in actively dividing cells can occur passively through a loss in activity or expression of DNMT1. Active or replication-independent DNA demethylation requires the presence of specialized enzymes (erasers) that modify methylated cytosine (5mC), which is then replaced by base excision repair pathway (BER) to native cytosine. Ten-eleven translocase (TET) enzymes modify 5mC to 5hmC by adding a hydroxyl group (Tahiliani et al., 2009). BER pathway then employs thymine DNA glycosylase (TDG) to cleave off and replace the modified cytosine with native cytosine (He et al., 2011).

Methylated DNA can be recognized by various proteins (readers), primarily preventing the binding of transcription factors and repressing transcription. Three classes of proteins, the MBD, UHRF, and zinc finger, are known to bind to the methylated cytosine of the DNA (L. D. Moore et al., 2013). Certain members of the MBD family of protein family such as MeCP2 can bind to the methylated DNA and further recruit other transcriptional repressor complexes (Nan et al., 1998)., The UHRF (ubiquitin-like, containing PHD and RING finger domain) family of proteins plays an important role in maintaining DNA methylation. They bind to DNMT1 during DNA replication and direct it to hemimethylated DNA (Bostick et al., 2007). The zinc finger

proteins bind to methylated cytosines via their zinc finger domain and repress transcription in a manner dependent on DNA methylation (H.-G. Yoon et al., 2003).

DNMTs regulate various physiological functions, and aberrant DNA methylation patterns are commonly observed in cancer. Genome-wide DNA hypomethylation and CpG island hypermethylation commonly occur in most cancer types. CpG islands that occur on gene promoters are unmethylated, while in cancer cells, various tumor suppressor genes are silenced by the hypermethylation of CpG islands. Retinoblastoma was the first tumor suppressor gene found to be silenced by hypermethylation of its CpG island (Greger et al., 1989). Since then, the CpG islands of various tumor suppressor genes (p53, MGMT, p16, RB) are hypermethylated in cancer (Esteller et al., 2000).

2.2.2 Histone modification

Histone acetylation is a reversible post-translational modification and occurs on various lysine residues of the histone tails. Histone acetylation is a mark for transcriptional activation, while deacetylation of transcriptional repression. The addition of the acetyl group to the lysine residues of histone tails is catalysed by enzymes known as histone acetyltransferase or lysine acetyltransferase (HAT/KAT). In humans, the histone acetyltransferases are grouped into three major families: GNAT, MYST, and p300/CBP (Kouzarides, 2007). Most HATs show activity towards more than one lysine, while some are highly specific. E.g., the H4K5 can be acetylated by the enzymes from P300/CBP, MYST family while H3K9ac can only be processed by acetyltransferase Gcn5/PCAF from the GNAT family (Kouzarides, 2007). Apart from the nucleus, certain HATs are also found in other cellular organelles, such as the cytoplasm or mitochondria. HATs are also involved in deacetylating non-histone substrates such as p53, BET proteins, α-Tubulin, ATM kinase, and transcription factors such as GATA1 (Shvedunova & Akhtar, 2022).

The enzymes that catalyse the removal of acetyl groups from histones are known as histone deacetylases (HDACs). In humans, 18 different HDACs are found, which are further grouped into two different families based on their catalytic mechanism. The first family consists of zinc dependent HDACs which require the binding of a zinc cation in their active site. They are further divided into three classes, class I (HDAC1, 2, 3 & 8), Class II (HDAC4, 5, 7, 9 and 6), and Class IV (HDAC 11) (Audia & Campbell, 2016). The second HDAC family is a nicotinamide adenine dinucleotide (NAD+) dependent and consists of class III HDACs (sirtuins). Sirtuins-mediated deacetylation is a two-step process where Sirtuins first cleave NAD, generating nicotinamide and ADP ribose. In the next step, Sirtuins transfer the acetyl groups from histones to the ADP ribose, generating acetyl-ADP-ribose (Park & Kim, 2020). Like histone acetyltransferases, histone deacetylases are also known to act on non-histone

substrates. HDAC1 is known to deacetylase lysine 382 of the tumor suppressor p53. Various other proteins, such as tumor suppressor ARID1A, transcription factor p65, and nucleophosmin, are substrates of HDACs (Shvedunova & Akhtar, 2022).

Histone methylation is another important post-translational modification that regulates the transcriptional process. Histone methylation occurs on the basic amino acid residues (lysine & arginine) of the histone tails. Lysine is known to be mono (me1) di (me2) or tri (me3) methylated, while arginine can be mono methylated (me1) or di methylated with the latter being either symmetrical (me2s) or asymmetrical (me2a) (Greer & Shi, 2012). The methylation of the histone lysine residues can either be activating or repressing. The methylation of lysine four of histone 3 (H3K4) is a mark for transcriptional activation. The monomethylated histone 3 (H3K4me) is unique to enhancer regions, while trimethylation (H3K4me3) occurs in promoter regions. Other activating lysine methylation marks are H3K36 and H3K79 and are found on gene bodies. The methylation of K9 and K27 of histone 3 is a marker for transcriptional repression. The arginine methylation of histone proteins is not very well characterized, and certain symmetric arginine methylations are known to be associated with transcriptional repression (H3R8me2s and H4R3me2s) (Jambhekar et al., 2019).

Histone methylation is regulated by a diverse group of enzymes that either add a methyl group on the lysine or arginine amino acids (methyltransferases/writers) or remove them (demethylases/erasers). Like the DNA methyl transferases, the histone methyltransferases acquire the methyl group from the universal methyl donor s-adenosylmethionine and transfer it to lysine or arginine of its substrate proteins. Histone methyltransferases are divided into three families: the SET-domain containing proteins, the DOT1L-like proteins that act on lysine, and the arginine N-methyltransferase (PRMT) family (Greer & Shi, 2012). All families of histone lysine methyltransferases (except for DOT1L) consist of a conserved SET domain, responsible for their catalytic activity. Methyltransferases are site-specific in adding the methyl groups on their substrates. E.g., DOT1L catalyses methylation of H3k79, PRC2 recognizes H3K27 and SETD1/MLL adds methyl groups on H3K4. The arginine methylation is catalysed by the protein arginine methyltransferase (PRMT) family, with PRMT1 being the major mammalian arginine methyltransferase acting on monomethylated arginine (MMA) and asymmetric dimethylated arginine (ADMA). The symmetric dimethylation of arginine is less frequent and catalysed by PRMT5 (Jambhekar et al., 2019). Histone demethylases are broadly divided into two families based on their catalytic activity, the amino oxidase domain and the jumonji C (JmjC)-domain-containing demethylases (Greer & Shi, 2012). The first histone demethylase discovered was the amino oxidase domain-containing demethylase KDM1A (Lysine-specific demethylase 1a) (Y et al., 2004). KDM1A is known to demethylate both mono and di-methylated lysine 4 of histone3 resulting in transcriptional repression (Y.-J. Shi et al., 2005). KDM1A has also

been shown to act as a transcriptional activator by catalyzing the demethylation of H3K9me (Metzger et al., 2005).

The modification of histone proteins relays the effect on various cellular processes by either directly modifying the chromatin structure or recruiting other regulatory proteins (readers) on the chromatin (Bannister & Kouzarides, 2011). For instance, adding or removing acetyl groups on proteins is sufficient to influence the protein-protein or protein-DNA interactions. The acetylation of lysine 16 of histone 4 (H4K16ac) is known to disrupt the internucleosomal interaction between the H4 tail and H2A-H2B acidic patch. The H4K16 tail region interacts with the acidic cavity formed by histone H2A and stacks the histone together in a tight configuration. Acetylation of the H4K16 results in disruption of histone 4 and histone H2A electrostatic interaction and unpacks the nucleosomes (Shvedunova & Akhtar, 2022). A specialized group of proteins recognizes histone modifications called the reader proteins. Acetylation marks are recognized by three classes of reader protein domains: bromodomain, the double PHD finger (DPF), and the double pleckstrin homology (PH) domain. BRD4, a Bromodomain-containing protein has been recently shown to recognize the acetylated histones at the enhancers and promote enhancer-activated transcription through RNA polymerase II (Narita et al., 2021).

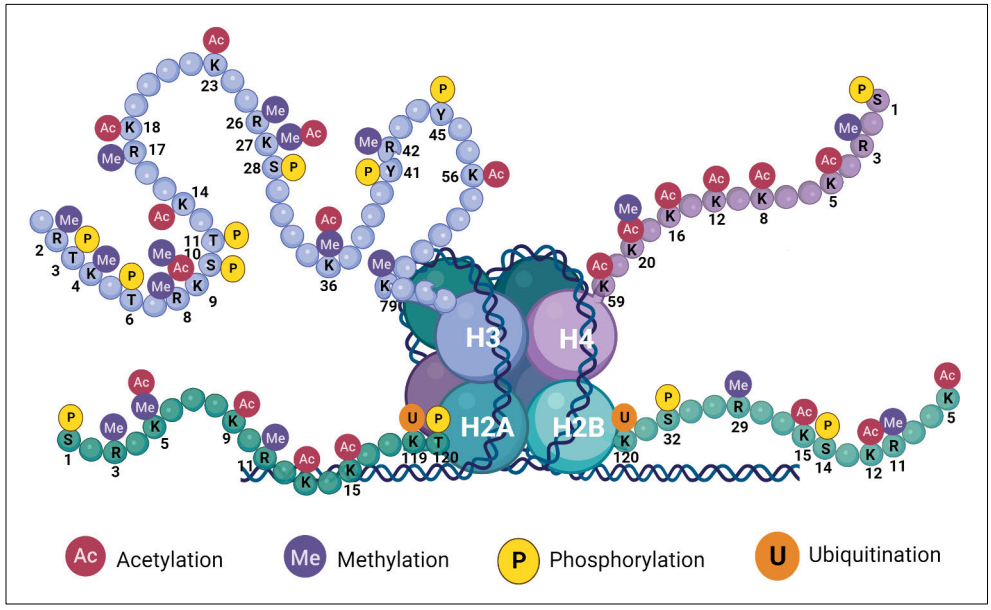

Figure 3. Sites of post translational modifications of Histone tails. Representation of the histone octamer (H2A, H2B, H3, and H4) wrapped around the DNA. The amino acid residues in the c-terminal tail of the histones are shown to be modified by various post-translation modifications (Ac = acetylation, Me = methylation, P = phosphorylation, U = ubiquitination). Created with BioRender.com.

More distinct classes of reader domains recognize protein lysine methylation as compared to acetylation or phosphorylation. These include the PHD finger, PWWP, ankyrin repeats, and the Tudor royal family of domains (chromodomains, Tudor, PWWP, and MBT) (Bannister & Kouzarides, 2011). The synergy between different histone modifications can regulate the binding of a reader domain-containing protein to the chromatin. The PHD finger domain-containing protein DPF3B is a part of the BAF chromatin remodeling complex. DPF3B can recognize the H3K14ac through its PHD domain and recruit the BAF complex to the chromatin, resulting in an open chromatin state. The methylation or acetylation of H3K4 reduces the interaction of the PHD domain with histones and further prevents the recruitment of the BAF complex (Zeng et al., 2010).

The histones can be modified by various epigenetic modifications such as acetylation and methylation, which can be interpreted by reader domain-containing proteins. This further leads to the recruitment of chromatin modifiers or other regulatory proteins, resulting in various biological outcomes.

2.2.3 Chromatin remodeling

The DNA is tightly wrapped around histone proteins in the nucleus, giving rise to compact structures called nucleosomes. Chromatin can exist in a highly condensed structure called heterochromatin or a lightly packed structure called euchromatin. Heterochromatin is inaccessible to transcriptional machinery due to its compact structure and is closed for transcription. Euchromatin can be assessed by the proteins controlling gene expression and is considered open for transcription. Chromatin remodeling is a process by which the chromatin structure is modified dynamically, giving rise to changes in chromatin's open and closed structures and is regulated by a specialized group of proteins (Wegel & Shaw, 2005; Nair & Kumar, 2012). Chromatin remodeling in mammals is catalysed by four different families of chromatin modelers: SWI/SNF, CHD, ISWI, and INO80 family (Längst & Manelyte, 2015). Chromatin remodelers are DNA translocases that use the energy generated by ATP hydrolysis to create a force to move and reposition the nucleosomes. All families of remodelers consist of a similar ATPase domain with helicase motifs but differ in specialized domains that read the histone modification (Nair & Kumar, 2012; Längst & Manelyte, 2015).

The SWI/SNF family of proteins is an evolutionarily conserved family of remodelers that were first discovered in yeast. The family consists of an actin-binding HSA (helicase-SANT) domain on their N-terminal and a bromodomain on their C-terminal. The bromodomain is responsible for recognizing lysine-acetylated histones. In humans, three different SWI/SNF complexes are formed by varying assembly of the complex members. Each of the complexes is composed of one of the

ATPase catalytic subunits, either BRG1 (SMARCA4) or BRM (SMARCA2), and a group of proteins called BAFs (BRG/BRM associated factors) that are involved in binding to DNA or proteins (Z. Li et al., 2023). SWI/SNF complexes associated with histone acetyltransferase are mainly associated with transcriptional activation and are known to be involved in DNA replication and DNA damage repair (Tyagi et al., 2016). Repressor activity of the complex has also been reported, where they have been shown to repress the activity of the E2F1 transcription factor (Trouche et al., 1997).

The chromodomain-helicase-DNA binding (CHD) chromatin remodellers are a family of nine proteins with a helicase-ATPase domain and a chromodomain that recognizes methylated histones (Watson et al., 2012). The CHD3/4/5 are distinct from other members of the groups in containing two PHD (plant homeodomain) zinc finger domains in their N-terminal that recognize the acetylated histones (Längst & Manelyte, 2015). The CHD family members can function independently or as a part of a large multiprotein complex regulating various biological outcomes. The CH7/8 associated complexes are found at gene enhancer and promoter regions resulting in transcriptional initiation and elongation while CHD3/4/5 are associated with the chromatin repressor complex (NuRD) (Murawska & Brehm, 2011; Bracken et al., 2019). The chromatin remodeling activity of the NuRD complexes is catalysed by one of the three CHD family members (CHD3/4/5), which function as the ATP-dependent helicase. The core of the complex is formed by the association between histone deacetylase (HDAC1/2) and the histone chaperone RBBP4/7. The complex interacts with the histone tails through the histone tail binding protein MTA1-3, while the DNA interaction is facilitated by methylated CpG binding proteins MBD2 or MBD3. The zinc finger containing the scaffolding protein GATAD2A/B bridges the MBD and the CHD subunits together. The NuRD complex regulates cellular processes such as cell differentiation, transcriptional repression, and regulation of DNA damage repair. The CHD4-containing NuRD complexes have also been shown to fine-tune the transcription by maintaining the nucleosomal density and controlling its access to transcription factors and RNA polymerases, thereby ensuring an appropriate transcriptional response (Bornelöv et al., 2018).

The ISWI family (Imitation SWItch) consists of an N-terminal ATPase domain catalyzing the chromatin remodeling and a C-terminal HAND-SANT-SLIDE domain, which binds to the DNA. The ISWI family members exist in various complexes that determine their involvement in multiple functions such as regulating nucleosomal assembly, chromatin structure, and DNA repair (Y. Li et al., 2021). Inositol requiring 80 (INO80) family consists of a split ATPase domain that scaffolds the RuvB helicase protein. They are involved in recombination, DNA repair, and nucleosome positioning and can catalyse the exchange of histone variants H2A-H2B to H2AZ-H2B (Längst & Manelyte, 2015).

Chromatin remodelers regulate various cellular processes like transcription control, DNA replication and repair, nucleosome assembly, and organization and regulation of chromatin access (Clapier et al., 2017). Changes in the association between complex members regulate diverse functions, while their dysregulation can lead to oncogenic transformation and other developmental disorders (Tyagi et al., 2016).

2.2.4 Post-translational modifications of epigenetic proteins

Epigenetic proteins regulate multiple cellular processes and can be regulated by various post-translational modifications (PTM) such as phosphorylation, acetylation, ubiquitination, etc. While the mechanism of action of the epigenetic proteins is extensively studied, very little is known about the PTM that regulates their activity.

Various high-throughput experiments have detected multiple phosphorylation changes in DNMT1, but very few have been functionally characterized (Jeltsch & Jurkowska, 2016). A few kinases are known to act on DNMT1 but none of the phosphatases that might be involved in regulating its activity have been identified. Phosphorylation of DNMT1 by AKT and PKC at serine 127/137 has been shown to disrupt its association with the DNMT1-PCNA-UHRF1 complex leading to global DNA hypomethylation and tumorigenesis (Hervouet et al., 2010). DNMT1 was shown to be regulated by two mutually exclusive PTM's: Methylation of lysine 142 by a methyltransferase SET7 that decreases its stability and phosphorylation of DNMT1 at S143 by AKT increasing its stability (Estève et al., 2011). Later studies found a lysine-specific demethylase (KDM1A) to remove the SET7-mediated methylation of DNMT1 (lysine 142) stabilizing its activity (J. Wang et al., 2009). DNMT1 is known to contain over 120 possible sites for lysine methylation and only lysine 142 has been identified to regulate the function of DNMT1. Of the various possible sites of acetylation in DNMT1 a few are known to be important for its function. HDAC1 was found to deacetylate and stabilize DNMT1 which was antagonized by TIP60-mediated acetylation resulting in the degradation of DNMT1 through the UHRF1-mediated ubiquitination (Du et al., 2010; Qin et al., 2011).

One of the major epigenetic complexes involved in gene repression is the Nucleosome Remodeling and Deacetylase complex (NuRD). Both the enzymatic activity and NuRD complex formation is regulated by various post-translational modifications. The NuRD functions by a combination of two different enzymatic activities, the ATP-dependent nucleosome remodeling through CHD3/4/5 and the deacetylation of histone tails through HDAC1/2. Though the NuRD complex is a transcriptional repressor, it has been shown that the PTM of specific components of the complex can change its role to be an activator (T. Yang et al., 2012). Acetylation of HDAC1 by Histone acetyltransferase has been shown to inhibit its deacetylase

activity and further dimerization and inhibition of HDAC2 (Y. Luo et al., 2009). Another member of the complex MTA1 is demethylated by another epigenetic eraser LSD1 resulting in its dissociation from the complex and further transcriptional activation (Nair et al., 2013). Phosphoregulation of the members of the NuRD complex is poorly understood. Only Casein kinase 2 has been shown to regulate the activity of both HDAC1 and HDAC2. Phosphorylation of HDAC1/2 by CK2 is important in maintaining its enzymatic activity and ability to form complexes (Pflum et al., 2001; Tsai & Seto, 2002). The role of phosphatases as well as the upstream signaling cascades regulating the phosphorylation events has never been addressed. The only known PTM regulating the Nucleosomal remodeling member of the complex CHD3 is sumoylation resulting in its dissociation from the chromatin and gene activation (Yamashita et al., 2016). Even though the PTM of a few complex members is shown to be important for its biological role very little is known about the PTM resulting in the assembly and chromatin recruitment of the NuRD complex members.

Recently, PTM of a few methyltransferases (writers) was found to be important in cancer progression and drug resistance. AKT was found to phosphorylate and downregulate the activity of epigenetic writer protein KMT2D. Drugs targeting PI3K-AKT pathways resulted in hyperactivity of KMT2D, resulting in a relaxed chromatin state that mitigated the effects of PI3K inhibition therapy in breast cancer (Toska et al., 2017). Another study showed that phosphorylation of EZH2 at serine 21 resulted in its inactivation and further hypomethylation of H3K27, leading to drug resistance in multiple myeloma (J et al., 2015). Another important methyltransferase involved in cancer is DOT1L. Despite various known PTMs on this 1403 amino acid long protein, minimal research investigating the role of these PTMs is available. A recent study found that the acetylation of DOT1L at lysine 358 prevents its RNF8-mediated ubiquitination, leading to DOT1 stabilization and further tumor growth and metastasis (C. Liu et al., 2020).

2.3 Epigenetics of lung cancer

Various genetic and epigenetic events contribute to the progression of lung cancer. Genetic aberration leading to the simultaneous inactivation of the tumor suppressor TP53 and RB is a common feature of SCLC. Among the various subtypes of NSCLC, LUSC shows frequent mutations in TP53 and RB while LUAD is linked to the mutations in multiple genes including KRAS, EGFR, NF1, KEAP1, STK11, and TP53. Similarly, the alterations in epigenetic pathways such as DNA methylation, chromatin organization, and histone modifications are hallmarks of lung cancer that lead to the growth and progression of various lung cancer subtypes (Langevin et al., 2015; Hoang & Landi, 2022).

Cancer cells frequently show alterations in DNA methylation patterns, leading to the suppression of tumor suppressors by DNA hypermethylation and activation of oncogenes by DNA hypomethylation (Shinjo et al., 2012; Witte et al., 2014). The DNA methyltransferases (DNMT1, DNMT3a, and DNMT3b) are found to be substantially expressed in lung cancers, causing hypermethylation of tumor suppressor genes, especially in smokers, and associated with poor prognosis (Lin et al., 2007; Liang et al., 2021). Various studies have reported that the maintenance DNA methyltransferase (DNMT1) is overexpressed in lung cancer (H. Kim et al., 2006; Lin et al., 2010). In vivo studies on a tobacco carcinogen-induced mouse model of lung cancer found a significant reduction in tumor growth upon DNMT1 inhibition (Belinsky et al., 2003). The siRNA-mediated depletion of DNMT1 in lung cancer cells led to promoter demethylation and expression of tumor suppressor genes p16(ink4A) and RASSFIA (Suzuki et al., 2004). Further knockdown of DNMT1 in lung cancer cells resulted in growth inhibition and induction of apoptosis in vitro and in vivo (Q. Lai et al., 2017). DNMT1 has been found to cooperate with DNMT3A in DNA methylation-mediated repression of tumor suppressor genes in cancer cells (Rhee et al., 2002). Human bronchial epithelial cells (HBEC) immortalized by the induction of hTERT/CDK4 showed an elevated expression of DNMT3B, which further led to an increased rate of transformation (Teneng et al., 2015). Another de novo methyltransferase, DNMT3A, has also been reported as a tumor suppressor. In a KRAS mutant mouse model, the deletion of DNMT3A promoted tumor growth (Gao et al., 2011) while another study found DNMT3A to be associated with a favourable prognosis in lung adenocarcinoma (Husni et al., 2016). Alterations in the DNA methyltransferase activity can lead to lung tumorigenesis through various mechanisms. In EGFR mutant NSCLC, DNMT1 promotes cellular proliferation by inducing promoter methylation and further downregulating the cell cycle suppressors (X.-Y. Wu et al., 2020). In cancer cells, DNA methylation can lead to silencing of tumor suppressor genes. The methylated gene promoter can be recognized by the MBD proteins that prevent the binding of the transcription factors to the DNA (Lopez-Serra et al., 2006). A more robust transcriptional silencing can be further achieved by recruiting the repressor complexes to the methylated DNA (Hoang & Landi, 2022).

Modifying histone tails by adding or removing various chemical groups is an important regulator of gene expression. Lung cancer cells show an abnormal pattern of histone H4 alterations, including loss of H4K20 trimethylation, hyperacetylation of H4K5/H4K8, and hypoacetylation of H4K12/H4K16 (Van Den Broeck et al., 2008). Histone-modifying enzymes and pathways are often dysregulated in cancer cells. Histone methylation is regulated by the synergy between specialized enzymes that either add a methyl group (methyltransferases) to the histone tail or enzymes that remove the methyl group (demethylases). The

dysregulation of histone methyltransferases impairs the regulation of various cellular processes such as DNA replication, repair, and gene expression and is commonly observed in various cancer types. Exome sequencing of lung tumors identified mutations in various methyltransferases, including the H3K79 methyltransferase DOT1L, which was found to be mutated in 3% of the lung adenocarcinoma samples (Campbell et al., 2016). The histone methyltransferase SETD2 catalyses the trimethylation at H3 lysine6 (H3K6me3), which is associated with gene bodies and is a marker for active transcription. Mutations in SETD2 leading to a loss in its methylation activity are found in various cancer types, indicating its role as a tumor suppressor (R. Chen et al., 2020). In a KRAS-driven mouse model of lung adenocarcinoma inactivation of SETD2 resulted in a loss of histone methylation (H3K36me3) leading to increased tumor growth and progression (Walter et al., 2017). Various histone demethylases are frequently overexpressed in lung cancer (Y. L. Chao & Pecot, 2021). The lysine-specific demethylase (KDM1A) catalyses the demethylation of H3K4 or H3K9, acting as both a transcriptional activator and repressor. KDM1A is found to be overexpressed in NSCLC, leading to an increased proliferation and migration of tumor cells (Lv et al., 2012). In NSCLC, KDM1A-mediated demethylation of H3K4me2 resulted in the repression of E-cadherin, leading to an increased epithelial-to-mesenchymal transition (Q. Liu et al., 2021).

The process of adding acetyl groups to the histone tails results in a transcriptionally open chromatin, while its removal results in a closed conformation of chromatin. Histone acetyltransferases (HAT) add an acetyl group to the chromatin, increasing its accessibility to transcriptional factors resulting in gene expression. Various members of the HAT family are found to be mutated in lung cancer (Y. L. Chao & Pecot, 2021). The histone acetyltransferase p300 was found to activate Snail and promote epithelial-to-mesenchymal transition (R. Chang et al., 2017). The removal of acetyl groups from the chromatin is catalysed by enzymes called histone deacetylase (HDAC), preventing its access to transcription factors and polymerases. Altered expression of the HDAC family members is commonly observed in lung cancer, leading to malignant transformation (Y. L. Chao & Pecot, 2021). Overexpression of HDAC6 in lung adenocarcinoma was associated with poor prognosis and mediated resistance against EGFR inhibition (Z. Wang et al., 2016). HDAC7 was identified as a tumor promoter in lung cancer, and the inhibition of HDAC7 suppressed the growth and proliferation of KRAS mutant lung cancer cells (Lei et al., 2017).

Chromatin remodeling complexes use ATP to disrupt the histone-DNA interaction and control DNA access to transcription factors and polymerases. Members of the chromatin remodeling complexes are frequently mutated in lung cancer. SMARCA4 is the ATPase encoding component of the SWI/SNF remodeling

complex and is one of the most commonly mutated genes in NSCLC (Medina et al., 2008). Immunohistochemical Staining of the primary lung adenocarcinoma cells showed frequent mutations in SMARCA4, while the loss of SMARCA4 was tumorogenic and correlated with poor prognosis in NSCLC (Reisman et al., 2003). Various studies have also reported the role of SMARCA4 as an oncogene where its inhibition sensitized NSCLC to CDK or aurora kinase inhibition (Tagal et al., 2017; Xue et al., 2019). Another member of the SWI/SNF chromatin remodeling complex, frequently mutated in lung cancer is ARID1A. Various loss of function mutations of ARID1A occur in lung cancer, resulting in tumor growth and progression (Jin et al., 2023).

2.3.1 Epigenetic biomarkers in lung cancer

One of the most crucial elements that determine the success and efficacy of cancer treatment is the diagnosis of the disease at an early stage. However, due to the lack of apparent early symptoms and precise screening results, a large number of lung cancer patients receive diagnoses when the disease has already spread to advanced stages (Y.-X. Shi et al., 2019). Recently, various studies have developed procedures to utilize non-invasive techniques to gather consistent and precise diagnostic biomarkers.

Exhaled air from the respiratory tract consists of vapors and aerosols, which can be condensed in a fluid form referred to as Exhaled breath condensate (EBC). Various studies have found macromolecules, including the genomic DNA in the EBC, giving rise to the possibility of analyzing the lung genome for disease-associated changes and diagnostic biomarker discovery (Sulewska et al., 2023). A study by Xiao and colleagues analyzed the EBC samples collected from NSCLC patients and found aberrant hypermethylation in the promoter of the tumor suppressor p16 gene (Xiao et al., 2014). Bronchial secretions contain a high number of epithelial cells, and a few studies have demonstrated their application in detecting the methylation of lung cancer-related genes. In one study, the bronchial secretions from NSCLC patients were assayed to successfully detect the hypermethylation of the tumor suppressor genes (PCDHGB6, HOXA9, and RASSF1A) in cancer cells (Ma et al., 2016). Peripheral blood is easier to collect and rich in genetic material, making it a preferred source of biomarker discovery. Hulbert et al isolated plasma and sputum from NSCLC patients and used the quantitative methylation-specific real-time PCR to detect changes in the methylation of genes associated with early-stage cancer risk (Hulbert et al., 2017).

Various studies have explored the feasibility of epigenetic changes as a prognostic biomarker in lung cancer. A study by Brock and colleagues addressed the association between gene methylation and the risk of tumor recurrence in NSCLC

patients. Seven candidate genes were analyzed for DNA methylation in the lymph nodes of NSCLC stage I patients using the methylation-specific PCR assay. The study reported that the altered promoter methylation of four genes (p16, CDH13, APC, RASSF1A) was associated with micrometastases and disease recurrence (Brock et al., 2008). DNA methylation can be associated with improved and shortened overall survival depending on the gene type. The hypermethylation of the DNA repair gene MGMT has been reported as a recurring event in many cancers, including the NSCLC (Esteller et al., 1999; Zöchbauer-Müller et al., 2001). In a genome-wide DNA methylation study in NSCLC patients, more than four hundred methylated genes were found to be associated with tumor cells. The study further predicted the methylation of HOXA2 and HOXA10 as a prognostic marker in squamous cell carcinoma where it was found to be associated with patients who relapsed (Heller et al., 2013).

Unlike DNA methylation, the application of Histones as cancer biomarkers is not extensively addressed. A few studies have analyzed the blood of cancer patients for circulating histones and their post-translational modification. In the event of cell death, the endonucleases cleave the nucleosome's linker DNA, breaking them into shorter fragments. These nucleosomal fragments can be detected in cancer patients' blood, indicating increased cell death during chemotherapy or radiotherapy (Holdenrieder et al., 2004). Circulating nucleosomes have also been analyzed for changes in post-translational modifications (PTM) in various cancer types. In one of the studies, the sera of breast cancer patients showed an elevated level of STAT2 on H3K9me3 and H4K20me4 while it was downregulated in the case of colorectal cancer (Leszinski et al., 2012). Immunostaining is widely used to detect the bulk changes in histone modification between healthy and tumor tissues. Changes in global histone patterns as a potential biomarker in NSCLC were analyzed using antibodies against the various PTM modifications on H3 and H4. Patient groups with increased histone acetylation were associated with a better prognosis, while those with an increased methylation showed poor prognosis (J. S. Song et al., 2012). Another study found that a decrease in H3K4me2 and H3K18ac in lung cancer patients correlated with a lower probability of survival (Seligson et al., 2009). Immunohistochemistry is easier to perform compared to sequencing studies. However, the disadvantages include the inability to detect gene-specific changes and the requirement of large amounts of patient materials.

To improve results and save the lives of people diagnosed with NSCLC, it is crucial to identify the most effective course of treatment. Alterations in epigenetic pathways leading to aberrant epigenetic modifications are common in various cancers including lung cancer. Changes in epigenetic events occur at the initial stages of the disease progression and can help in early cancer diagnosis improving the therapy response.

2.3.2 Epigenetic targeting in lung cancer

Therapeutic targeting of epigenetic proteins can potentially overcome drug resistance in different cancer types. DNA methylation inhibitors have shown promise when combined with chemotherapeutic agents, primarily by demethylation-mediated activation of tumor suppressors. Several studies have found an increase in DNA methylation upon drug treatment to be associated with resistance to therapy response (Nyce, 1997). A study compared cisplatin-resistant and sensitive NSCLC cells for differentially methylated genes and found that the promoter of insulin-like growth factor-bindin protein-3 (IGFBP-3) to be hypermethylated in cisplatin-resistant cells (Ibanez de Caceres et al., 2010). In NSCLC cell lines, the DNMT1 inhibitor azacitidine, when used in combination with cisplatin or gemcitabine showed a high synergy and a rapid decrease in cellular proliferation (Füller et al., 2015). DNA hypermethylation has also been associated with targeted therapy response (Chao & Pecot, 2021). A study found that DNMT1 inhibition could overcome resistance to EGFR inhibitors in lung cancer cells. The oncogenic EGFR gene is hypermethylated in various lung cancer cell lines, increasing resistance to the EGFR inhibitor gefitinib. Chemotherapeutic drugs decitabine and azacitidine are hypomethylating agents and are routinely used for the treatment of acute myeloid leukemia. Treating resistant cells with the DNMT inhibitor decitabine resulted in the demethylation of the EGFR promoter, increased mRNA and protein expression, and sensitized the cells to gefitinib treatment (X.-Y. Li et al., 2013). Another study reported that the DNMT inhibitor azacytidine treatment increased survival of NSCLC patients (Momparler & Ayoub, 2001). Azacitidine-mediated DNA demethylation was found to upregulate immunomodulatory pathway genes such as PD-L1 (Chao & Pecot, 2021). DNMT inhibitors, together with immune checkpoint inhibitors, are currently under investigation in various phases of clinical trials of NSCLC, e.g., azacitidine in combination with pembrolizumab (NCT02546986) durvalumab (NCT02250326) and decitabine in combination with nivolumab (NCT02664181) (Al-Yozbaki et al., 2022; Y. L. Chao & Pecot, 2021).

Pharmacological inhibition of HDAC results in direct gene effects by overcoming HDAC-mediated gene repression and upregulation of tumor suppressor genes. The specificity of HDACi ranges from pan-HDAC inhibitors to class-specific inhibitors. The FDA has approved HDAC inhibitors for treatment in hematological malignancies but have failed to elicit an effective response in solid malignancies. Monotherapy using HDAC inhibitors in NSCLC was largely unsuccessful due to a weak safety profile and cytotoxicity; however, combination therapy using HDACi has shown promising results. Lung cancer with mutations in the KRAS or EGFR gene eventually develops resistance against a wide range of inhibitors and chemotherapy. In recent studies, HDAC inhibition has shown promise in overcoming the resistance to targeted therapy in NSCLC. EGFR mutant NSCLC cell lines resistant to osimertinib treatment were sensitized by combination therapy with

the HDAC inhibitor vorinostat (Tanimoto et al., 2017). In a mouse model of KRAS mutant NSCLC, the resistance against the MEK inhibitor trametinib was overcome by the inhibition of HDAC3 (Eichner et al., 2023).

Enzymes catalyzing histone post-translational modifications have emerged as an attractive drug target in the past decade. An orally available inhibitor of the lysine-specific methyltransferase KDM1A (GSK2879552) showed potent antitumor activity in a panel of SCLC cell lines in vitro and in vivo (Mohammad et al., 2015). The reader of the acetylated lysine BRD4 is a member of the BET protein family, and its overexpression positively correlates with an increased invasion and metastatic potential of NSCLC (Liao et al., 2016).

The past few decades have greatly advanced our understanding of epigenetic processes. Various epigenetic drugs have been discovered and are currently undergoing clinical trials. However, epigenetic monotherapy in solid cancers has not yet shown much promise. For example, decitabine and azacitidine are the most thoroughly researched DNMT inhibitors and are approved for the treatment of leukemia, but neither has shown promise in NSCLC monotherapy (Y. L. Chao & Pecot, 2021). One reason why earlier clinical trials did not work is that the amounts were too high; using DNMTi may need to be adjusted to a dosage that can cause DNA demethylation but does not kill healthy cells (Chao & Pecot, 2021). The use of epigenetic drugs in combination with chemotherapy or targeted therapy has shown promising results. Further understanding of epigenetic processes and their dysregulation in diseases will significantly improve epigenetic therapy in cancer.

2.4 RAS as an oncogenic GTPase

Jennifer Harvey discovered the RAS (rat sarcoma) gene as a retroviral oncogene in 1964. Harvey observed that newborn rodents, when injected with the preparation of murine leukemia virus derived from a leukemic rat, develop sarcoma (Harvey, 1964; Malumbres & Barbacid, 2003). The virus was termed Harvey murine sarcoma virus (Ha-MuSV). Following the previous discovery, another transforming virus causing lymphomas in rats was identified by W H Kirsten and called the Kirsten murine sarcoma virus (Ki-MuSV) (Kirsten & Mayer, 1967). Using nucleic acid hybridization, it was later identified that the genome of the sarcoma virus contained a sequence from rat DNA, indicating that the transforming potential of the virus arises from the recombination between the viral and rat genomes (Scolnick et al., 1973).

Both viral oncogenes encoded a 21-kDa protein responsible for their transformation potential, and the viral gene that encoded this protein was called v-ras (T. Y. Shih et al., 1979; Coffin et al., 1981). Later, the rat sarcoma DNA fragment homologs were identified in rats and later in humans (DeFeo et al., 1981). In mammals, the viral oncogene homolog of the Harvey sarcoma virus was termed H-

ras, while that of the Kirsten sarcoma virus was termed K-ras (Karnoub & Weinberg, 2008). The third member of the RAS family was discovered and cloned in human neuroblastoma cells and was termed N-ras (Hall et al., 1983).

2.4.1 RAS structure and downstream signaling

RAS signaling is activated by the binding of growth factors to the extracellular receptor tyrosine kinases. RAS proteins activate various signaling pathways by further interacting and activating their effector proteins, leading to cellular growth and proliferation. RAS proteins contain an effector domain recognized by their interaction partners, which in turn possess a RAS binding domain (RBD) (Rajalingam et al., 2007). Computational studies have predicted the human genome to contain at least 56 proteins that contain the RAS binding domain (RBD), of which eight bind with high affinity, 25 with low affinity, and four are predicted not to bind RAS (Kiel et al., 2021).

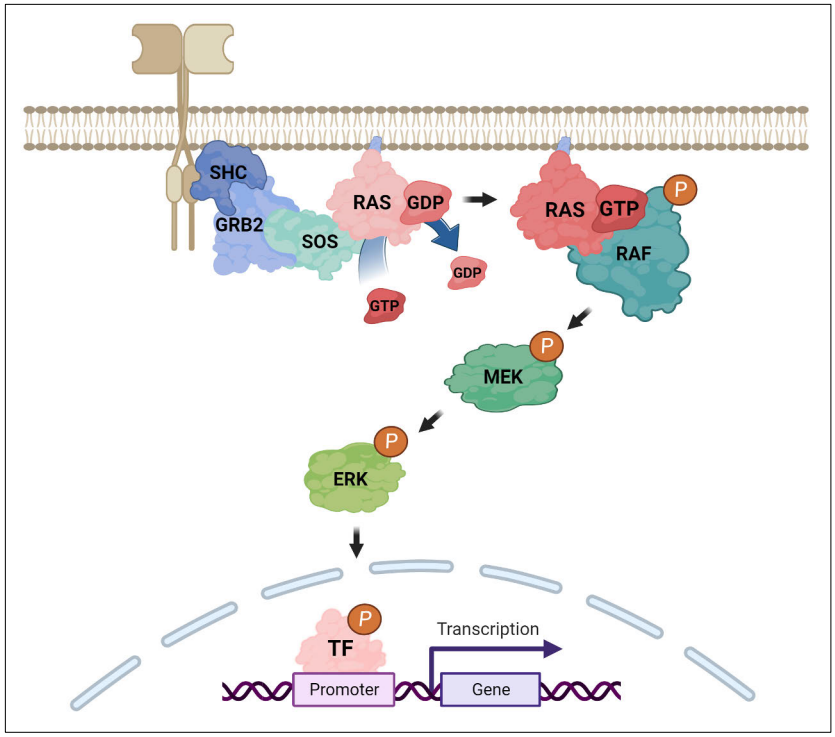

Figure 4. RAS-MAPK pathway. Binding of a growth factor to the extracellular domain of a tyrosine kinase receptor results in auto-phosphorylation and activation of the receptor. This facilitates the binding of adaptor proteins SHC and GRB2 to the receptor. The adaptor proteins recruit SOS, a guanine nucleotide exchange factor that activates RAS by facilitating the exchange of GDP to GTP. Active RAS binds and activates RAF, which further activates a downstream cascade of kinases, resulting in the phosphorylation of multiple proteins. Created with BioRender.com

The first RAS effector identified was the RAF kinase, followed by RAL (guanine nucleotide dissociation stimulator) and phosphatidylinositol 3-kinase (PI3K) (Moodie et al., 1993; Hofer et al., 1994; Rodriguez-Viciana et al., 1994).

RAS proteins are structurally organized into a N-terminal G domain and a C-terminal hypervariable region (HVR) (Hobbs et al., 2016). The G-domain consists of the switch I and II that bind the GTP/GDP and regulates the interaction between RAS and its effectors (Rajalingam et al., 2007; Buhrman et al., 2011). G-domain is followed by a highly variable region which undergoes various post-translational modifications and plays an important role in the cellular localization and trafficking of the RAS proteins. Synthesis of RAS proteins occurs in the cytoplasm and is followed by a series of post-translational modifications, which results in its membrane targeting and anchorage (Rajalingam et al., 2007).

Once anchored in the membrane, RAS proteins alternate between a GTP-bound active and a GDP-bound inactive state. Binding of GTP, leads to a conformational change in the switch regions keeping it in an active position, while the hydrolysis of the GTP to GDP alters the conformation and relaxes the switch regions, inactivating the RAS protein (Vetter & Wittinghofer, 2001). This cycling of RAS from an inactive GDP bound to an active GTP bound state occurs in response to extracellular stimuli and is regulated by a group of specialized proteins. The guanine nucleotide exchange factors (GEF) act as an activator of RAS by promoting the exchange of GDP to GTP. The binding of the GEF alters the conformation of the switch region, releasing the bound GDP from RAS and favouring the binding of GTP. The GTP binding releases the GEF from RAS, resulting in RAS activation and further interaction with its effectors (Rajalingam et al., 2007).

Upon the completion of the signaling cascade, RAS proteins are inactivated by the hydrolysis of GTP to GDP. RAS proteins have a low intrinsic GTPase activity and require GTPase activating proteins (GAP) for efficient GTP hydrolysis. The GAP proteins bind to RAS, accelerating their intrinsic GTPase activity by approximately 100,000-fold (Gideon et al., 1992). Mutations in the amino acid residues G12, G13, and Q61 prevent the binding of GAP to RAS leading to a constitutively active RAS leading to oncogenic signaling (Scheffzek et al., 1997).

2.4.2 RAS activation in cellular transformation

The role of RAS in human cell transformation began with the work of Weinberg and colleagues, who showed that the mouse fibroblasts (NIH3T3) could be transformed by introducing DNA isolated from the chemically transformed

mouse (C. Shih et al., 1979). This was followed by various studies showing that mouse cells can also be transformed by introducing the DNA isolated from non-virally induced human cancer cells (Krontiris & Cooper, 1981; Murray et al., 1981; Perucho et al., 1981). Soon, multiple studies reported that the genes isolated from human tumors that can transform NIH3T3 cells are a homolog of the retroviral oncogene discovered by Harvey in 1964 (Der et al., 1982; Parada et al., 1982; Santos et al., 1982; Cox & Der, 2010). Sequencing of the human cancer cell lines revealed point mutations in RAS proteins and was associated with the increased transformation potential of the oncogenic RAS (Capon et al., 1983; Cox & Der, 2010).

However, the widely perceived concept of RAS as an independent driver of cellular transformation was challenged when various studies showed that the introduction of RAS oncogene alone could not transform the primary embryo fibroblasts. It was found that, unlike the mouse fibroblasts that are transformed in a single step of RAS introduction, the RAS-mediated transformation of primary cells is a multistep process and requires additional factors. The mouse fibroblasts were shown to be transformed either by immortalizing them before RAS introduction or co-transfecting an additional oncogene (myc, polyomavirus large-T antigen) together with RAS (Newbold & Overell, 1983; Land et al., 1983).

Further work by Weinberg and colleagues showed that the RAS-mediated transformation of human cells requires additional factors compared with mouse cells (Figure 5). Human fibroblasts and epithelial cells were transformed by the introduction of the mutant HRAS together with the catalytic subunit of the human telomerase reverse transcriptase enzyme (hTERT) and the simian virus large T (LT) and small T (ST) antigens. The simian virus LT antigen inhibited tumor suppressor retinoblastoma (RB) and p53, while the ST inhibited the PP2A. Inhibition of PP2A by ST was found to be a prerequisite for transformation, as the modulation of the remaining factors could only immortalize the cells (Hahn et al., 1999, 2002).

The first mutation found in human cancer was reported in the RAS gene in 1984. The mutation was identified as a point mutation (G to C) in the first exon of the KRAS gene, resulting in the substitution of arginine in place of glycine as the twelfth amino acid of the KRAS protein. The mutation was limited to lung carcinoma and was absent in adjacent noncancerous cells (Santos et al., 1984). Since then, the cancer genome has undergone extensive sequencing, leading to the identification of over 500 cancer genes; however, mutations in RAS family genes still account for most mutations in cancer(Cox et al., 2014).

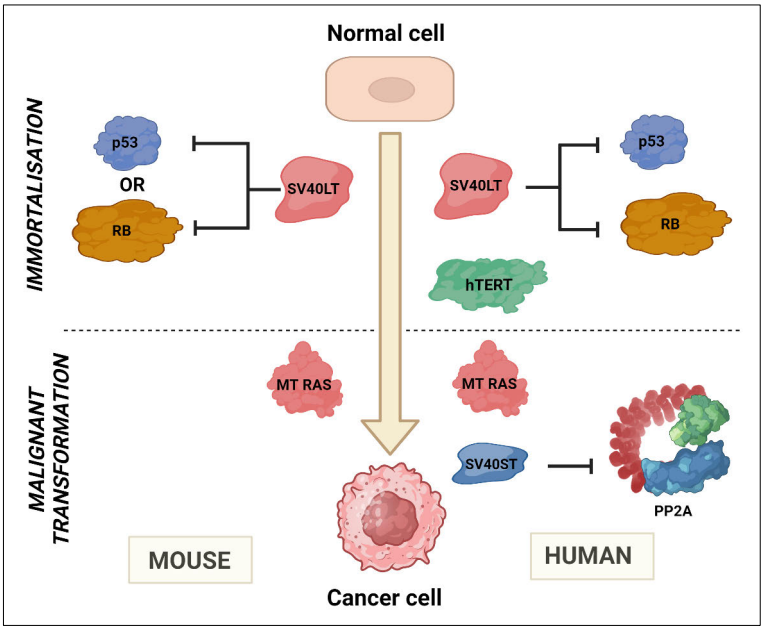

Figure 5. A schematic comparison between the cell transformation requirements in mouse and humans. Mouse cells can be Immortalised by inhibition of one of the tumor suppressors and further transformed by the expression of mutant RAS. On the other hand, transformation of human cell requires modulation of five cellular pathways, including the inhibition of three tumor suppressors. Created with BioRender.com

KRAS is the most frequently mutated RAS isoform in human cancers (85%), distantly followed by NRAS (11%) and HRAS (4%). Around 27% of human cancers show mutations in genes coding for one of the RAS isoforms however this varies among the cancer type for e.g, KRAS gene shows 97.7% mutation frequency in pancreatic ductal adenocarcinoma while is rarely mutated in Skin cutaneous melanoma (0.8%) (Cox et al., 2014)The mutational profile of the RAS isoforms varies depending on the cancer type. The KRAS isoform is most frequently mutated in pancreatic, lung, and colorectal cancers, with a nearly 100% frequency in lung adenocarcinoma (LAC) and pancreatic ductal adenocarcinoma (PDAC). Colon and rectal carcinomas (CRC) show a majority of KRAS mutations (86%) and a low frequency of NRAS mutations (14%), while the mutant HRAS has not been identified in CRC. NRAS is the most frequently mutated isoform in melanoma and acute myeloid leukemia, whereas HRAS mutations predominantly occur in bladder and head and neck squamous carcinomas (Cox et al., 2014; Hobbs et al., 2016).

A significant share (98%) of these mutations comprises G12, G13, and Q61, collectively termed the RAS hotspot mutations. The RAS isoforms show varying frequencies of these hotspot mutations among themselves. The KRAS gene is most frequently mutated at G12 (83%) and offers a very low frequency of Q61 mutations

(2%). In contrast, the NRAS gene is mostly mutated at Q61, while HRAS shows a similar frequency to the three hotspot mutations (Cox et al., 2014; Hobbs et al., 2016). Also, the type of hotspot mutation within an isoform varies between cancer types. E.g., KRAS G12 mutations mainly occur in PDAC (Pancreatic ductal adenocarcinoma), whereas G13 mutations occur in colorectal adenocarcinoma (CRC). The Q61-mutated NRAS primarily occurs in melanoma, while the G12 occurs in acute myeloid leukemia (Hobbs et al., 2016). The amino acid changes at the hotspot mutation site of the RAS residue also vary depending on the tumor type. In PDAC and CRC, the most frequent substitution at G12 is G12D, then G12V, while G12C is uncommon, whereas most G12 substitution in NSCLC is G12C.

The missense mutation at the hotspot codons can give rise to six different amino acid substitutions. However, the RAS isoforms are quite specific regarding the presence of amino acids. KRAS (41%) and NRAS (52%) show a higher preference toward G12D amino acid substitution, while HRAS mutations predominantly show G12V. G13D is the most prevalent replacement at the G13 codon for KRAS (89%) and NRAS (50%); however, it is uncommon in HRAS (3%), where G13R (85%) is the most common mutation. Furthermore, at codon Q61, Q61H is the most common KRAS mutation (58%), while it is uncommon in both NRAS (6%) and HRAS (5%), where Q61R is the most common replacement (47% and 43%, respectively) (Hobbs et al., 2016).

RAS hotspot mutations have been extensively characterized for their functional outcomes. Using NMR profiling, it was found that the G12 mutation results in a decreased sensitivity to RAS inactivation by GAP-mediated GTP hydrolysis, as seen in G12D and Q61K mutations, while in the case of the G12V mutation, there was a complete resistance against GAP activity. The mutants also showed a remarkable difference in their activation by GEF, where the G13D mutation led to a rapid exchange of GDP to GTP followed by Q61L. activation compared to the WT RAS, the G12V mutant showed a slower activation rate, but its activation state was maintained by complete resistance to GTP hydrolysis (Smith et al., 2013; Hobbs et al., 2016). The effect of different hotspot mutations in RAS exerts heterogeneous effects on effector interaction and downstream signaling. The KRAS G12C mutant showed decreased activation of AKT and increased activation of Ral, whereas the other KRAS mutants showed increased phosphorylation of AKT (Ihle et al., 2012).

2.4.3 RAS mediated epigenetic regulation

RAS-mediated downstream signaling is involved in regulating various epigenetic processes. The first indication of the role of RAS signaling in regulating the DNA methylation pathway came from the study that showed that the activation of RAS signaling results in the induction of DNMT1 promoter. The RAS effect was shown

to be mediated through the transcription factor AP-1, which had recognition sites in the 5′ upstream regions of DNMT1 (Rouleau et al., 1995). A follow-up study by the same group using RAS-amplified adrenocortical tumor cells (Y1) and showing a DNA hypermethylation phenotype further established the role of RAS in DNA methylation. The transfection of Y1 cells with the inhibitor of active RAS (GAP) resulted in a loss of cellular transformation of Y1 cells and a decreased AP-1 binding to DNMT1. GAP expression further reduced DNMT1 enzymatic activity, leading to global DNA hypomethylation. The introduction of the mutant HRAS resistant to GAP-mediated GTP hydrolysis resulted in retransformation of Y1 cells and restored the global DNA hypermethylation (MacLeod et al., 1995). A contrasting effect of RAS in regulating the global DNA methylation from the one shown in Y1 cells was also observed in the first study. It was seen that the expression of RAS in embryonic carcinoma cells (p19) resulted in an increased expression of DNMT1 mRNA; however, the patterns of global DNA methylation showed an opposing trend, resulting in DNA hypomethylation (Rouleau et al., 1995). Another study published the same year also shared a similar observation where the expression of RAS resulted in global DNA demethylation in mouse embryonal cells (Szyf et al., 1995). The two opposing effects on the global DNA methylation levels upon RAS expression are indicators that RAS activity might be involved in regulating both DNA methyltransferases and demethylase activity, which might further depend on various stages of cellular development and differentiation.

Promotor methylation of tumor suppressors resulting in their inactivation is frequently observed in cancer (Esteller, 2003). Various studies addressed the role of oncogenic RAS signaling and DNA methylation-mediated gene silencing. The association between the oncogenic KRAS mutations and the inhibition of tumor suppressors p16 by DNA methylation was first observed in colon cancer. It was found that the KRAS mutant colon cancer cells show frequent methylation of the p16 gene. Further, the KRAS-induced cellular transformation in immortalized cells showed an increased DNMT1 activity and further methylation and inactivation of the p16 gene (Guan et al., 1999). Several studies have shown that RAS upregulated the expression of DNMT1, but the mechanism behind the activation is currently unknown. The role of RAS in directly regulating DNMT1 phosphorylation has yet to be established.

Post-translational modifications of histones are an essential mechanism that regulates gene expression. Histones can be regulated by various PTMs, such as acetylation, methylation, and phosphorylation (Audia & Campbell, 2016). Histone acetylation is catalysed by the enzymes that either add (acetylase) or remove (deacetylase) the acetyl group on the histone tails (Audia & Campbell, 2016). Various studies have found the involvement of the RAS pathway in regulating histone acetylation. HDAC4 was identified as a downstream target of the RAS-

MAPK pathway and phosphorylation of HDAC4 by ERK1/2 resulted in its nuclear localization (X. Zhou et al., 2000). RAS has not been shown to regulate the phosphorylation of HDAC1 directly, however, a study showed RAS signaling lead to an increased interaction between HDAC1 and transcription factor SP1 leading to histone deacetylation (H.-C. Chang et al., 2004). RAS has been shown to regulate the activity of various histone acetyltransferases (HAT). RAS-PI3K signaling was shown to degrade the histone acetyltransferase p300-CBP, leading to a decrease in the H3K56 acetylation mark, while another study found that the RAS-induced transformation increased the activity of HAT transferases TIP60 and CBP (Y. Liu et al., 2012; Sánchez-Molina et al., 2014). RAS-mediated HAT activation leads to a global increase in the acetylation of H4K8ac and H4K12ac in RAS-transformed cells, increasing the global chromatin accessibility. Recent studies have found that oncogenic HRAS(G12V) activates the expression of the transcription factor GATA4 by an increase in H3K27ac marks at GATA4 enhancers (Nabet et al., 2015). Another study compared the three RAS isoforms for activating the target genes of the transcriptional factor SRF. The RAS isoforms showed high specificity in gene activation, and the oncogenic NRAS(G12N) specifically increased the acetylation of histone lysine 9 and 23 (H3K9/23ac), leading to the activation of SRF targets (Yi et al., 2018). The role of RAS in regulating the enzymes catalyzing the histone methylation first surfaced with studies showing an increased expression of histone demethylase KDM6B by the RAS signaling. RAS pathway recruited KDM6B to the INK4a/ARF locus and demethylation of H3K27me3 both in mouse and human fibroblasts. However, the study failed to identify the specific phosphosites on the histone-modifying enzymes to be regulated by the RAS pathway (Barradas et al., 2009; Agger et al., 2009). A study published in 1991 by Mahadevan and colleagues first described the phosphorylation of histone H3. Stimulation of oncogenic signaling pathways by treating the cells with growth factors or phosphatase inhibitors led to the rapid phosphorylation of multiple serine residues of histone H3(Mahadevan et al., 1991). Later studies revealed the involvement of the RAS-MAPK pathway in the phosphorylation of histone H3 at serine 8 and 28, which is further found to be elevated in RAS-transformed cells and associated with chromatin relaxation and transcriptional activation (Thomson et al., 1999; Strelkov & Davie, 2002; Dunn & Davie, 2005; Dunn et al., 2009). Together with H3, the RAS-transformed fibroblasts also show an increased H1 phosphorylation, leading to chromatin opening and gene expression (Chadee et al., 1995; Taylor et al., 1995).

Phosphorylation of proteins regulating various epigenetic processes is essential for various functions such as cellular localization, enzymatic activity, chromatin recruitment, and complex formation (Treviño et al., 2015). The role of RAS proteins in regulating the epigenetic modifications or the enzymes catalyzing these modifications is not extensively established. Earlier studies in the first half of 1990

identified the RAS pathway in directly regulating the phosphorylation of histones. Later studies showed the RAS-mediated downstream signaling to be important for regulating various epigenetic processes such as DNA methylation and histone acetylation. However, very few epigenetic regulatory proteins are known to have a phosphosite directly regulated by the RAS pathway. For example, of the various histone deacetylases in humans, only HDAC4 is known to contain a phosphosite regulated by the RAS pathway. Various studies associate RAS with the regulation of DNA methylation, but none of the phosphosites on the enzymes catalyzing the DNA methylation are regulated by RAS activity.

2.5 PP2A a tumor suppressor phosphatase

PP2A (protein phosphatase 2A) is a major human serine-threonine phosphatase. PP2A acts as a tumor suppressor, and its activity is inhibited during cancer progression mainly by the expression of its inhibitory proteins, such as CIP2A, PME-1, and SET. PP2A inhibition results in the activation of various growth promoting pathways such as oncogenic MAPK/ERK and PI3K/AKT/mTOR pathways or inhibition of the tumor suppressive p53 pathway (H.-H. Li et al., 2007; Y.-C. Kuo et al., 2008a; Wlodarchak & Xing, 2016). Recent findings have found PP2A to be involved in regulating various cellular pathways, such as the regulation of RNA biogenesis, maintenance of nuclear lamina and cellular cytoskeleton, and DNA damage (Kauko et al., 2020a; Laine et al., 2021). PP2A was shown to regulate the phosphorylation of splicing factors such as HNRNPA1 and RBM4. The subcellular localisation of the RNA binding protein NPM was shown to be regulated by PP2A, with the dephosphorylated NPM1 predominantly translocated to the nucleus (Kauko et al., 2020a).

The mitogen activated protein kinase (MAPK) pathway is primarily involved in cellular proliferation. Initial studies related to the relationship between MAPK pathway and PP2A revealed that PP2A dephosphorylates and inhibits the activity of both MEK MAPKK and ERK MAPK (Anderson et al., 1990; Gómez & Cohen, 1991). For example, the simian virus 40 small t antigen mediated PP2A inhibition resulted in the activation of MEK and ERK leading to cell proliferation. At the same time, no effect was seen on the activity of RAF (Wlodarchak & Xing, 2016). Later, the B56 containing PP2A holoenzyme was found to dephosphorylate ERK and inhibit its activity (Alessi et al., 1995; Letourneux et al., 2006). The other members of the RAS signal transduction pathway inhibited by the PP2A-mediated dephosphorylation include MEK activating RAF-1 kinase or SRC homology-2-containing protein (SHC) (Dent et al., 1995; Ugi et al., 2002). The role of PP2A as an activator of kinase was first found when PP2A was shown to activate RAF-1 kinase by dephosphorylation of its serine 259 (Abraham et al., 2000). PP2A activity can also negatively regulate the RAS pathway upstream of these kinases through the

dephosphorylation and stabilization of SPROUTY2, which further inhibits the formation of the RAS complex (Lao et al., 2007). Overall, the PP2A activity is associated with the negative regulation of the MAPK pathway by dephosphorylation and inactivation of various proteins in the cascade.

PI3K/AKT/mTOR is another commonly disrupted pathway in cancer. The oncogenic kinase AKT can be dephosphorylated at Thr308 by PP2A holoenzymes containing PR55α, inhibiting its activity (Y.-C. Kuo et al., 2008a). PP2A is involved in a cross talk with the AKT downstream target GSK-3β. PP2A dephosphorylates GSK-3β at serine 9 increasing its kinase activity, resulting in the GSK-3β mediated inhibition of PME-1 and increased methylation of the C subunit of PP2A (Y. Wang et al., 2015). PP2A inhibits mTOR-mediated translation initiation by dephosphorylating the mTOR downstream substrates. PP2A dephosphorylates and activates 4E-BP1, which is a translation inhibitor, while inhibiting the p70S6 kinase that initiates translation (Peterson et al., 1999; Janssens & Goris, 2001).

PP2A also regulates the activity of the tumor suppressive p53 protein. PP2A complex containing B56γ subunits dephosphorylate the Thr55 residue of p53 in response to DNA damage. This stabilizes p53, increasing apoptosis (H.-H. Li et al., 2007). The downstream target of p53 is another tumor suppressor protein, Retinoblastoma (RB), which binds to the transcription factor E2F1, leading to cell cycle arrest (Ross et al., 1999). Phosphorylation of RB prevents its interaction with E2F1 while its dephosphorylation by PP2A results in RB-E2F1 complex formation further leading to growth arrest (Magenta et al., 2008). PP2A can also regulate various proteins involved in the apoptotic pathway. PP2A activation by ceramide resulted in the formation of B56 containing holoenzymes which, upon translocation to mitochondria, dephosphorylated and inactivated BCL2. Inhibition of PP2A using okadaic acid prevented the dephosphorylation of BCL2 and increased cellular proliferation (Ruvolo et al., 2002). PP2A-mediated dephosphorylation of the Bcl-2 family member and proapoptotic protein BAD led to increased cellular apoptosis (Chiang et al., 2001).

Overexpression of PP2A inhibitory proteins such as CIP2A, SET, and PME-1 is commonly observed in cancer cells and is responsible for PP2A inactivation and cellular transformation (Kauko & Westermarck, 2018; Haanen et al., 2022). PME-1 was first purified from a bovine brain and found to remove the methyl group added by PTPA to the Leu309 of the PP2A-C subunit (J. Lee et al., 1996; Ogris et al., 1999; De Baere et al., 1999). An additional mode of PME-1 mediated PP2A inactivation was proposed by analyzing the crystal structure of PME-1 in complex with PP2A. The structure revealed that PME-1 binds to an active site of the AC dimers and evicts the Mn2+ ions from the PP2A active site (Xing et al., 2008). A recent study found PME1 to interact with the different B65 regulatory subunits of PP2A, catalyzing the demethylation of the C subunit (Y. Li et al., 2022). PME-1 mediated inhibition of PP2A activity has been shown to promote tumorigenesis in various cancer types, whereas its

inhibition resulted in abrogation of oncogenic signaling (Aakula et al., 2023; Kaur et al., 2016; Pusey et al., 2016; Puustinen et al., 2009; Wandzioch et al., 2014).

Another nuclear inhibitor of PP2A, SET, was identified in AML as a fusion partner of oncogene CAN and found to localize predominantly in the nucleus (Y. Adachi et al., 1994). A recently published NMR study found SET to specifically inhibit the B56γ subunit of PP2A (De Palma et al., 2019). SET overexpression occurs in many cancer types and is involved in regulating various oncogenes and tumor suppressor genes and cellular pathways such as RNA biogenesis and chromatin organisation (Yuan et al., 2017; Kauko et al., 2020a; Di Mambro & Esposito, 2022). For example, SET inhibition in pancreatic cancer cells resulted in an increased PP2A activity and degradation of oncogenic MYC further inhibiting the proliferation of pancreatic cancer cells (Farrell et al., 2014). On the other hand, in medulloblastoma, SET mediated inhibition of PP2A further inhibited the TP53 function through increased phosphorylation of TP53 inhibitor MDM2 (Wei et al., 2019).

CIP2A (cellular inhibitor of PP2A) was identified as a major cytoplasmic inhibitor of PP2A in human malignancies and has been shown to promote oncogenesis by preventing MYC degradation (Junttila et al., 2007). CIP2A expression is seen to be elevated in the majority of cancers and is a major driver of oncogenic signaling pathways such as KRAS, MYC, and E2F1 and regulates various cellular processes such as cellular organization, biogenesis, and DNA repair (Kauko et al., 2020a; Laine et al., 2021, 2013a). A detailed mechanism of CIP2A mediated PP2A inhibition was recently shown by Pavic et. al. The study found that CIP2A directly binds to the PP2A-B56α trimer, displacing the A subunit and forminga CIP2A-B56α-PP2Ac complex. Further CRISPR mediated mutagenesis of a CIP2A amino acid residue in the interaction domain inhibited MYC expression and reduced in vivo tumor growth (Pavic et al., 2023).

2.5.1 PP2A structure and substrate specificity

PP2A is a heterotrimeric protein complex consisting of a 65 KDa scaffolding A (PP2A-A/PR65) subunit a 36 KDa catalytic C (PP2A-C) subunit, and a regulatory B subunit. The A subunit, through its scaffolding activity brings the B and C subunits together forming the active holoenzyme complex (Xing et al., 2006; Xu et al., 2006). The B subunits regulate the cellular localisation and substrate specificity of PP2A, while the C subunit catalyses the dephosphorylation of its substrate (Cho & Xu, 2007; Xu et al., 2008; Flegg et al., 2010). The C subunit is methylated at Leucine 309 by enzyme LCMT1 (leucine carboxyl methyltransferase) and this methylation facilitates the binding of the B subunit and formation of the trimeric PP2A holoenzyme complex in cells. Another major factor affecting the holoenzyme assembly is the phosphorylation of tyrosine 307 of the C terminal tail, which prevents the recruitment

of several members of the B subunit family (Longin et al., 2007). The A and B subunits show weak binding together and, hence, do not form a stable complex. The BC interaction stabilizes the B subunit binding to the complex interfaces. B subunits shield most of the C subunit and dictate the holoenzyme complex's cellular localization and substrate binding specificity (Cho & Xu, 2007). For instance, the B subunits have been shown to regulate the cellular localization and substrate specificity of PP2A holoenzyme in the brain. The Bα/β were found to be cytosolic, whereas BϒΥ was found to be associated with cytoskeletal fractions (Strack et al., 1998). Another study showed that the B56α, β, and ε isoforms contain a nuclear export signal (NES) and are mainly cytoplasmic, while B56 Δ and ϒ do not have the NES and are localized to the nucleus. Further, B56α promoted the nuclear export of the C subunit and centrosome targeting of the A subunit (Flegg et al., 2010).

Recently the subunit-specific substrate recognition of PP2A was established by the finding that B56 recognizes its substrates through a short linear motif (SliM) LxxIxE (Hertz et al., 2016; X. Wang et al., 2016). Additionally, the B56 substrates were found to contain a positively charged region (basic patch) adjacent to the SLIM, which interacted with the negatively charged residues (acidic patch) on B56 and further stabilized this interaction (X. Wang et al., 2020). A study recently identified a B55α specific binding motif important for substrate recognition and binding (Fowle et al., 2021).

2.5.2 PP2A inhibition in cellular transformation

Since PP2A regulates various oncogenic and tumor suppressive mechanisms, its inhibition is paramount in achieving cellular transformation. Rangarajan and colleagues showed that inhibition of tumor suppressor p53 and activation of the oncogenic RAS signaling pathway could readily transform mouse cells. Transforming the human cells is more complex, and in addition to inhibiting p53 and RB and expression of hTERT, and mutant RAS, requires the inhibition of tumor suppressive function of PP2A (Hahn et al., 1999, 2002; Rangarajan et al., 2004).

The inhibition of PP2A activity in oncogenic transformation occurs by various mechanisms such as mutations or post-translational modification of its subunits or expression of its inhibitory proteins (Ruediger et al., 2011; Kauko & Westermarck, 2018). Cancer cells require the activity of PP2A for cell cycle progression and a complete inactivation of PP2A is lethal. Hence, the mutations in PP2A subunits are not very common. As a result, cancer cells have created a variety of strategies to precisely regulate PP2A activity in their favor. PP2A is predominantly inhibited in cancer cells through the expression of its inhibitory proteins such as CIP2A, PME-1, and SET (Kauko & Westermarck, 2018; Haanen et al., 2022). PP2A inhibition is important phenomena in all tumor types irrespective of the mutation status.

The first indication of PP2A as a tumor suppressor occurred when its inhibition by okadaic acid was found to promote tumor growth (Fujiki & Suganuma, 1993). Further, the small tumor antigens (ST) of polyoma and simian virus were found to interact and block the PP2A heterotrimeric complex formation. This inhibited the phosphatase activity of PP2A as indicated by an increased phosphorylation of PP2A target substrates (S. I. Yang et al., 1991). Later, the DNA sequencing of the primary tumors and the derived cell lines revealed various mutations in the A subunit of PP2A and some were found to functionally disrupt the PP2A complex formation (S. S. Wang et al., 1998; Ruediger et al., 2001). William C. Hahn and colleagues further showed that suppressing the Aα activity by 50% of the endogenous PP2A results in cellular transformation (W. Chen et al., 2005). Later, Sablina and colleagues identified that the knockdown of specific PP2A subunits (B56α, B56γ, and PR72/130) can replace the need for expression of SV40ST antigen in human cell transformation (Sablina et al., 2010).

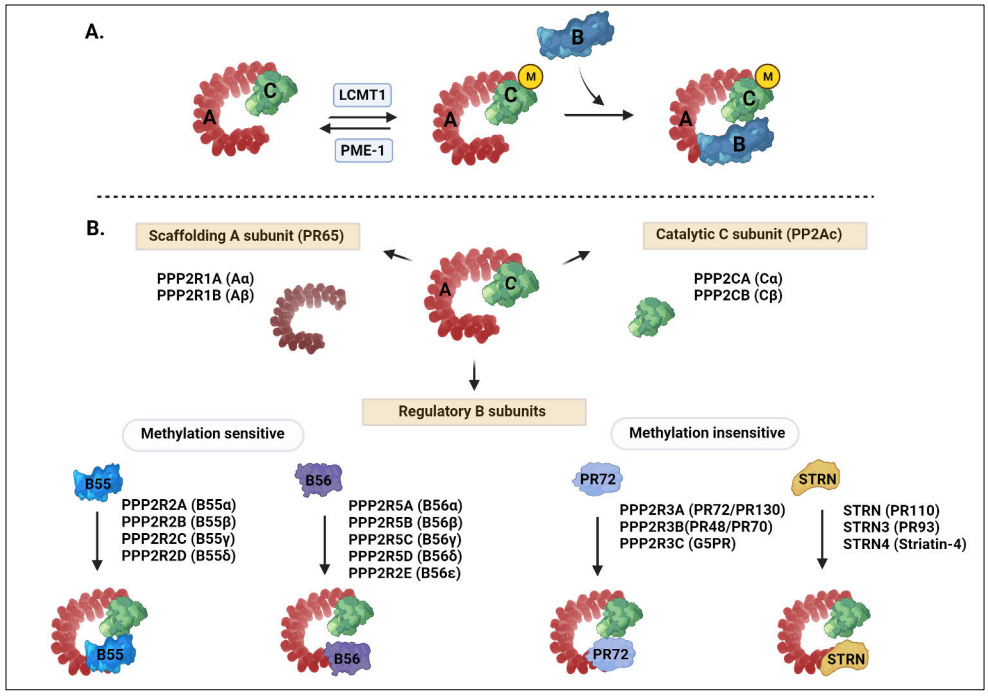

Figure 6. Regulation of the PP2A holoenzyme complex formation. **A**. LCMT1 methylates the C-subunit of the PP2A resulting in the formation of the heterotrimeric holoenzyme complex. PME-1 inhibits PP2A complex formation by preventing the methylation of the C-subunit. **B**. The PP2A dimer, consisting of A and C subunits, can incorporate various B subunits, giving rise to the active holoenzyme complex. Each of the PP2A subunits has multiple isoforms that fit together in different combinations and enable PP2A to catalyse a wide range of substrates specifically. Created with BioRender.com.

Mutations affecting PP2A subunits are not commonly found in cancer, and PP2A inactivation is mainly by non-genomic mechanisms (Kauko & Westermarck, 2018). The A subunit (PP2R1A1) is the most frequently mutated among all the PP2A subunits. Various studies have focussed on the role of the A subunit mutations on cell signaling pathways. The mouse models of the A subunit point mutation E64D showed reduced binding with B′α and B′δ subunits in lung and brain tissue and further induced lung cancer in mice upon benzopyrene treatment (Ruediger et al., 2011). Overexpression of the mutant A subunit in endometrial cancer cells resulted in increased tumor growth coupled with increased phosphorylation of PP2A substrates (Haesen et al., 2016). Another study showed that the R183W mutation of the A subunit annulled the tumor suppressive function of PP2A and imparted resistance against MEK inhibition (O'Connor et al., 2020).

The B subunits of PP2A show much lower mutation rates than the A subunit. Truncation of the B56Y1 subunit in mice resulted in malignant growth, while decreased expression of B55α in AML resulted in hyperphosphorylation of its substrate (T309 of AKT) (Ito et al., 2000). In cancer cells, modulation of the B subunits is also known to occur through Epigenetic mechanisms such as hypermethylation of B55β promoter (Tan et al., 2010) or micro-RNA binding to B55δ mRNA, leading to a decreased expression (Zhuang et al., 2016).

The C subunit of PP2A is not mutated frequently but can be dysregulated in cancer through post-translational modifications and by expression of PP2A inhibitory proteins. The amino acids ^{304}TPDYFL^{309} at the tail region of the C subunit constitute a significant target of post-translational modifications. Various kinases are known to phosphorylate the Tyr307 C subunit of PP2A in vitro, resulting in a loss of its enzymatic activity and increased phosphorylation of cellular proteins (J. Chen et al., 1992; Guo & Damuni, 1993). The unphosphorylated form of PP2A showed increased interaction with SRC and JAK2 kinases and negatively regulated their activity (Yokoyama & Miller, 2001; Yokoyama et al., 2003). The methylation at Leu309 of the C subunit of PP2A is required for proper holoenzyme assembly and is regulated by the enzymes PME1 (protein phosphatase methylesterase-1) and LCMT1 (leucine carboxylmethyltransferase-1) (Kaur & Westermarck, 2016; Westermarck & Neel, 2020).

2.5.3 PP2A reactivation as a therapeutic strategy in cancer

The first evidence of PP2A activation as a therapeutic strategy to treat cancer began with the discovery of ceramides as PP2A activators. Ceramides are a family of sphingolipid metabolites found as cytosolic PP2A activators in rat glioma cells. It was further established that the presence of B subunits in the PP2A heterodimer is essential for PP2A activation and the dissociation of the B subunits from the

heterodimer abolished the activation of PP2A (Dobrowsky et al., 1993). More than a decade later PP2A inhibitor SET was found as a direct target of ceramides. Ceramides were shown to bind to SET inhibiting the PP2A and SET interaction and leading to MYC degradation in A549 lung cancer cells (Mukhopadhyay et al., 2009). However, ceramides are also known to influence the immune response, hence its effect on PP2A activation could be related to alternate pathways.

A few years after the discovery of ceramides as PP2A activators, Kunitomo Adachi et. al. synthesized an immunosuppressant (FTY720) by modifying a compound (ISP-1) isolated from an insect eating fungus *Isaria sinclairii* (K. Adachi et al., 1995). In line with its immunosuppressant role, FTY720 has been approved by the FDA for the treatment of multiple sclerosis (Strader et al., 2011). The first evidence of FTY720 as an anticancer agent appeared when it was shown to induce apoptosis in prostate cancer cells (J. D. Wang et al., 1999). The antitumor effects of FTY720 were further established from studies showing it ,inhibits FAK, decreases pERK in prostate cancer cells, induces apoptosis, and reduces metastasis in mouse breast cancer models (Azuma et al., 2002; Permpongkosol et al., 2002). PP2A activation as a mechanism for FTY720 was first proposed by Matsuoka and colleagues who showed that FTY720 treatment results in the activation of the purified PP2A (J. D. Wang et al., 1999). Also, the leukemic cells treated with FTY720 showed AKT dephosphorylation independent of the PI3K pathway, indicating its direct regulation by PP2A (Matsuoka et al., 2003). It was later found that FTY720 binds to SET like ceramides and leads to a decrease in tumor growth in lung cancer mouse xenografts (Saddoughi et al., 2013).

Trifluoperazine hydrochloride (TFP), an FDA-approved antipsychotic and antiemetic (dopamine receptor antagonist), belongs to the family of phenothiazines. It was initially identified as a nuclear export inhibitor of FOXO1 transcription factor in a drug screen. Treatment of cells with TFA resulted in nuclear retention of FOXO1 through calmodulin inhibition (Kau et al., 2003). It was later found that Phenothiazines exert their anti-cancer property by directly targeting PP2A, and shRNA-mediated silencing of PP2A subunits alleviated phenothiazine's response towards tumor growth (Gutierrez et al., 2014). To further refine the activity of phenothiazines as anticancer drugs, they were engineered to abolish the antipsychotic property while retaining the PP2A interacting function. This led to generating small molecule activators of PP2A (SMAPs) (Kastrinsky et al., 2015). DTO-61 acts as a molecular glue and binds at a pocket defined by the interface between the PP2A-A subunit, B56α, and the methylated C subunit (Leonard et al., 2020). This leads to the stabilization of the PP2A heterotrimeric complex and further substrate dephosphorylation.

Various studies have established the PP2A mediated role of SMAPs as an anticancer compound in different cancer types, including the highly oncogenic

KRAS and MYC driven cancers. SMAPs were, for the first time, shown to inhibit the growth of KRAS mutant lung cancer cells in mouse xenograft models. It was initially proposed that SMAPs target the Aα subunit of PP2A, driving conformational changes and further activation (Sangodkar et al., 2017). SMAPs were shown to abrogate the MEK inhibitor resistance in KRAS mutant cells via PP2A activation (Kauko et al., 2018). PP2A activation through SMAP was shown to overcome MYC mediated mTOR inhibition resistance. SMAP mediated PP2A activation in PDAC cells decreased MYC expression, which synergized with mTOR inhibition, resulting in reduced tumor growth of PDAC cells. It was recently found that SMAPs significantly penetrated the blood brain barrier and showed superior efficacy in killing patient-derived glioma cells compared to various kinase inhibitors (Merisaari et al., 2020).

A recent approach of targeted dephosphorylation involving recruiting a phosphatase to a specific target phosphoprotein resulting in precise substrate dephosphorylation has been demonstrated. Targeted dephosphorylation was first shown to catalyse the dephosphorylation of AKT by PP1 using a heterobifunctional molecule containing binding sites for both PP1 and AKT (Yamazoe et al., 2020). To degrade the hyperphosphorylated tau proteins in Alzheimer's disease, Zheng et. al. developed a DEPhosphorylation Targeting Chimaera (termed as DEPTAC) which facilitates the recruitment of PP2A to tau proteins leading to tau dephosphorylation and degradation (Zheng et al., 2021). However, the peptide chimeras in the above studies showed dephosphorylation phenotypes at very high concentrations and are susceptible to proteolytic degradation. Further, their high molecular weight and large structure account for poor cellular penetration and difficulty crossing the blood-brain barrier. A recent study has tried to overcome these problems by developing small molecule-based phosphorylation targeting chimera (PhosTACs) for targeting tau proteins (Hu et al., 2023). The targeted dephosphorylation approach has many advantages, as it provides high selectivity and prevents off-target effects. The phosphorylation targeting chimeras is still in developmental stages and holds a promising future.

2.5.4 PP2A mediated regulation of epigenetic machinery

PP2A has been shown to promote DNA hypomethylation through the ERK/AKT pathways in T-cells. Inhibition of the PP2A C subunit enhanced AKT and ERK phosphorylation, leading to increased DNMT1 expression and DNA hypermethylation (Sunahori et al., 2013). TET2 is an enzyme that converts 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), leading to DNA demethylation and mutations disrupting TET2 enzymatic activity that has been shown to promote myeloid tumorigenesis (Ko et al., 2010). Phosphorylation of TET2

at Serine 99 promotes its stabilization and further increases the global 5-hydroxymethylcytosine (5hmC) levels. PP2A complex consisting of the B55α subunit was found to dephosphorylate TET2 at S99 and destabilize it. The S99 region of TET2 is prone to mutations, and its dephosphorylation by PP2A points towards an oncogenic role of PP2A (Kundu et al., 2020). In line with the oncogenic role of PP2A in TET2 regulation, the PP2A inhibitor LB100 is undergoing clinical trials for Myelodysplastic syndrome, which shows frequent TET2 mutations (NCT03886662).

The first evidence of regulation of protein arginine methylation by PP2A came from the work of Duong FH and colleagues while studying the hepatitis C virus (HCV) induced protein expression in patient samples of chronic hepatitis. The samples showed an increased level of PP2A while reduced levels of STAT1 methylation at arginine 31 (Duong et al., 2004). The follow-up study found that an arginine methyltransferase PRMT1 was responsible for the demethylation of STAT1. The PP2A mediated PRMT1 inactivation led to an increased helicase activity of the viral NS3, while PP2A inhibition by OA treatment inhibited the replication of the viral replicons (Duong et al., 2005). A decade later, PP2A was found to dephosphorylate PRMT1 at Serine 297, thereby inhibiting the arginine methylation activity of PRMT1 (Zhao et al., 2019). PRMT1 is an arginine specific methyltransferase catalyzing the arginine methylation of histone four (Strahl et al., 2001). Despite its role as a tumor suppressor in HCV mediated hepatocarcinoma, PRMT1 has been shown to activate oncogenic pathways including EGFR and inhibit tumor suppressors such as p53 (L.-M. Liu et al., 2021; Suresh et al., 2022). Another arginine based methylatransferase, PRMT5, is regulated by PP2A. PRMT5 is mainly involved in gene repression and is proposed to be a therapeutic target in KRAS mutant colorectal cancer (Shifteh et al., 2020). In adult T-cell leukemia (ATL), PP2A dephosphorylates PRMT5 at Serine 355, inhibiting its oncogenic methyltransferase activity (Ichikawa et al., 2020). PRMT5 negatively regulates PP2A in glioblastoma, and combined inhibition of both PRMT5 and PP2A reduces glioblastoma tumor growth (Otani et al., 2021).

BRD4 (bromodomain-containing protein 4) belongs to the BET family of proteins that bind to acetylated histones and maintains chromatin structure (R. Wang et al., 2012). The binding of the epigenetic reader BRD4 to acetylated histones is regulated by casein kinase 2 mediated phosphorylation (S.-Y. Wu et al., 2013). PP2A was identified as a phosphatase against BRD4 in TNBC. PP2A inhibition led to increased phosphorylation and chromatin recruitment of BRD4, leading to resistance to bromodomain inhibitors. Activation of PP2A using perphenazine synergized with BETi JQ1 in triple negative breast cancer while further downregulation of PP2A led to resistance against BET inhibitor JQ1 (Shu et al., 2016; Kauko et al., 2020a). Another study showed that the overexpression of the PP2A inhibitor SET correlates

with the high phosphorylation status of BRD4 in the TNBC patient cohort and is a predictor of poor outcomes (Sanz-Álvarez et al., 2021).

Histone deacetylases remove the acetyl group from histones and play an important role in transcriptional regulation. PP2A has been shown to regulate the cellular localization of class IIa HDACs extensively (HDAC4 & 5, 7). Class IIa family of HDACs shuttle between the nucleus and cytoplasm through a unique C terminal domain containing binding sites for 14-3-3 proteins (Park & Kim, 2020). The binding of 14-3-3 proteins to HDAC4 promotes its cytoplasmic export, and this shuttling event has been shown to regulate transcription through chromatin remodeling (McKinsey et al., 2000, 2001; A. H. Wang & Yang, 2001). PP2A enables the nuclear import of HDAC4 by dephosphorylating binding sites of 14-3-3 proteins and S298 leading to gene repression (Paroni et al., 2008). In p53 deficient tumor cells, HDAC4 is associated with the PP2A complex containing B56α subunits and regulates chromosomal segregation. The siRNA mediated inhibition of either HDAC4 or the B56α subunit of PP2A resulted in chromosomal segregation defects and mitotic arrest (Cadot et al., 2009). The therapeutic relevance of PP2A mediated nucleo-cytoplasmic shuttling of HDAC4 was uncovered in glioblastoma. PP2A inhibitor PME-1 was found to promote kinase inhibitor resistance in glioblastoma by promoting the cytoplasmic localization of HDAC4 (Kaur et al., 2016).

HDAC5 was found to be associated with B55α containing PP2A complex in cardiomyocytes. PP2A mediated dephosphorylation at Ser259/Ser498 of HDAC5 led to its nuclear import and further transcriptional repression (Sucharov et al., 2006; Weeks et al., 2017). PP2A was shown to control the repressor activity of HDAC7 by dephosphorylating the 14-3-3 binding sites in HDAC7, leading to its nuclear import. Chaperone protein 14-3-3 prevented the dephosphorylation of these sites by binding and masking them from PP2A activity (Martin et al., 2008). In another study, the Bα subunit of PP2A was found to be an essential regulator of cytoskeletal dynamics by regulating HDAC7 cellular localization. The dephosphorylating activity of PP2A resulted in the nuclear localization of HDAC7 and transcriptional silencing, while the Inhibition of PP2A Bα or HDAC7 leads to disruption of the microtubule network (Martin et al., 2013). Among the class I HDACs (HDAC 1,2,3,8), only HDAC2 is known to interact with PP2A directly. The Association of PP2A with other families of HDACs is currently unknown.

The PP2A inhibitor SET is part of the inhibitor of acetyltransferases (INHAT) complex, which binds to histones, masking them from acetylation and further leading to transcriptional repression (Seo et al., 2001a). Contrary to the above findings, a study showed that SET initiates transcription from chromatin templates and acts as a transcriptional activator (Gamble et al., 2005). The INHAT complex containing SET was found to inhibit the acetylation of p53, repressing its activity and increasing cellular proliferation (J.-Y. Kim et al., 2012). SET was further found to prevent DNA

demethylation and promote gene silencing (Cervoni et al., 2002). However, another study showed that SET overexpression leads to DNA hypomethylation (Almeida et al., 2017). The role of PP2A in the regulation of epigenetic proteins is poorly understood. Of many epigenetic complexes involved in gene regulation few are known to be direct targets of PP2A. PP2A is a major S/T phosphatase and the is involved in the regulation of the majority of the oncogenic signaling pathways. Epigenetic machinery is known to be heavily modulated by cancer cells in their favor, and the role of PP2A in regulating these proteins needs to be further investigated.

3 Aims

Protein Phosphatase 2A (PP2A) is a human tumor suppressor that mostly downregulates various signaling pathways through dephosphorylation activity. All cancer types irrespective of their mutation status requires the suppression of PP2A. PP2A activity is required by cancer cells for cell cycle progression; hence, a complete inhibition of PP2A is lethal. PP2A is mainly inactivated in cancer through the non-genomic mechanism by expression of its inhibitory proteins CIP2A, PME-1, and SET. On the other hand, RAS activates various kinases downstream of its signaling pathway, leading to cell proliferation. Various RAS protein mutations result in the RAS signaling pathway's hyperactivation, which drives oncogenic growth. It has been very well established that RAS-mediated human cell transformation requires the simultaneous inhibition of PP2A. However, whether the PP2A inhibition and RAS hyperactivity converge in regulating unique phosphorylation sites during oncogenic transformation is unclear. My thesis aims to address the unsolved question of the convergence of RAS and PP2A activities on protein phosphorylation and the cellular pathways affected by it. The specific aims of the thesis were as follows:

1. To understand the role of the phosphorylation events coregulated by RAS and PP2A on the function of proteins regulating epigenetic processes.
2. To characterize and compare the RAS and PP2A regulated transcriptome.
3. To compare and characterize the roles of PP2A inhibitors PME-1 and SET in gene regulation.

4 Materials and Methods

4.1 Cell culture

All cells were cultured in a humified incubator with 5% CO2 and a temperature of 37 °C. Cells were cultured in media as per their growth conditions and split once they reached a confluence of around 75%. Cells were frequently tested to confirm negative mycoplasma contamination.

4.2 Transfections

Gene silencing was performed using the Lipofectamine™ RNAiMAX Transfection Reagent (#13778150) for 72 hours while protein overexpression was done using the jetPRIME® transfection reagent Polyplus (#114-15) for 48 hours according to the manufacturer's protocols.

4.3 Western blotting

For western blotting analysis, plates containing the cells were washed twice with ice-cold PBS. Cells were lysed directly on the plates by adding the RIPA buffer supplemented with protease and phosphatase inhibitors. Cells were collected by scrapping and incubated on ice for 15 minutes. In between incubation, cells were vortexed twice at maximum for 5 seconds. Lysates were then sonicated using the highest pulse at ± 30 s for five minutes. Tubes were then vortexed vigorously for 10 seconds and then centrifuged at the highest speed for 20 minutes at 4 °C. Supernatant-containing proteins were collected in precooled fresh tubes. The protein concentration was determined using the BCA assay using Pierce™ BCA Protein Assay Kits (#23225). Protein concentration was then normalized, and proteins were subjected to denaturation using the 6X loading buffer while boiling at 95 °C for 10 minutes. Lysates were then loaded on 4–20% Mini-PROTEAN® TGX™ Precast Protein Gels Biorad (#4561093), and proteins were separated at 80–100 V until the bromophenol dye reached the bottom of the gels. Gels containing the separated proteins were assembled on Trans-Blot Turbo Mini 0.2 µm PVDF Transfer Packs Biorad (#1704156) and blotted in Trans-Blot Turbo Transfer System (#170-4155)

using a mix molecular weight program for 5 minutes. Membranes containing the blotted proteins were then blocked by incubating them in 5% milk in a TBST buffer for 1 hour, and gently shaking them at room temperature. Membranes were then subjected to the respective primary antibodies and incubated overnight at 4 °C with shaking. Membranes were washed in TBST buffer thrice for five minutes each and subjected to secondary antibodies labeled with HRP and diluted to 1:5000 in TBST for 1 hour at RT. Membranes were washed in TBST thrice for 5 minutes each, and 1 ml of Pierce™ ECL Western Blotting Substrate (#32106) was added to the membranes, and the reaction was allowed to proceed for two minutes at RT. The signal was developed using the Bio-Rad Laboratories ChemiDoc Imaging Systems.

Table 1. List of antibodies used.

ANTIBODY NAME	DILUTION	CATALOGUE NO.
FLAG	1:1,000	F3165
GAPDH	1:10,000	5G4-6C5
GFP	1:5000	sc-9996
HDAC1	1:5,000	ab7028
HDAC2	1:1,000	sc-9959
H3	1:5,000	sc-374669
PME-1	1:5,000	sc-20086
SET	1:2,500	sc-133138
PPP2R1A	1:2,500	sc-15355
PAN-RAS	1:5,000	OP40-100UG

4.4 Pull down assay

To determine the protein-protein interaction between B56α and HDAC1 or RNF168 and TP53BP1, pull-down assays were used. NCI-H460 cells were seeded in 10 cm plates at a density of 1 million cells per plate. The next day, the cells were transfected with the respective plasmids using the jetPRIME® transfection reagent Polyplus (#114-15). Forty-eight hours before transfections, cells were harvested by scraping on ice and subject to lysis using the buffer containing 100 mM NaCl, 1 mM MgCl2, 10% glycerol, 0.2% protease inhibitor tablet (Roche), and 25 units/ml Benzonase (Millipore). Tubes were incubated for 15 minutes with rolling at 4 °C, and then the concentration of NaCl and EDTA was further increased to 200 and 2 mM, respectively, and the tubes were additionally incubated for 10 minutes with rolling at 4 °C. The lysates were then centrifuged at maximum speed and maximum speed,

and from the supernatant, 10% of the lysate was collected in fresh tubes. In contrast, the remaining supernatant was used for immunoprecipitation. Lysates were added to the tubes containing the prewashed ChromoTek GFP-Trap® Agarose (#gta-20) or ChromoTek DYKDDDDK Fab-Trap™ Agarose (#ffa) beads. Protein interactions were facilitated by rotating the tubes for 2 hours at 4 °C. Beads were washed thrice in lysis buffer and boiled at 95 °C in 2X lysis buffer. Protein interactions were determined by western blotting.

4.5 Subcellular protein fractionation

Cell fractionation was used to estimate the cellular localization of various proteins. Cells were fractioned using the Subcellular Protein Fractionation Kit for Cultured Cells from Thermo Fisher Scientific (#78840) as per the manufacturer's instructions. Before fractionation, the respective buffers were supplemented with protease inhibitors provided in the kit. Cells were first harvested using trypsinization, then washed in PBS twice, then washed in PBS twice, and 1 million cells were used for fractionation. To fractionate the cytoplasmic fractions, cells were suspended in 100 µl of cytoplasmic extraction buffer (CEB) and then incubated with gentle mixing for 10 minutes at 4° C. Post-incubation cells were centrifuged at 500g for 5 minutes, and the supernatant containing the cytoplasmic fractions were collected in new tubes and stored at -80° C until further use. Next, the membrane-bound proteins were from the pellets from the previous step. 100 µl of membrane extraction buffer (MEB) was added to the tube with the cell pellet, and it was vortexed at the highest speed for 5 seconds and then incubated at 4 °C with gentle mixing. Tubes were centrifuged at 3000g for 5 minutes, and the supernatant containing the membrane-bound proteins was collected in new tubes and stored at -80 °C. Pellets containing the nucleus were further processed to separate the nucleoplasmic proteins with the chromatin-associated proteins. Pelleted nuclei were first washed in PBS, then suspended in 50 µl of nuclear isolation buffer (NEB), and vortexed for 10 seconds at maximum speed. Tubes were then incubated for 30 minutes at 4° C with rotation. Tubes containing the lysed nuclei were centrifuged at 5000g for 10 minutes to pellet out the chromatin, while the nucleoplasmic proteins in the supernatant were collected in fresh tubes. The chromatin was then treated with 50 µl of nuclear isolation buffer (NEB) supplemented with 150U of micrococcal nuclease (MNAse) and 5 mM calcium chloride and vortex at maximum speed for 10 seconds. Tubes were then incubated at room temperature (RT) for 15 minutes and vortex again for 15 seconds at the highest speed. Tubes were centrifuged at 16,000g for 10 minutes, and the supernatant containing the chromatin-associated proteins was transferred to new tubes while the pellets were treated with pellet isolation buffer (PEB) to recover the

cytoskeletal fractions. Tubes were incubated for 10 minutes at 4 °C and centrifuged for 10 minutes at maximum speed to recover the cytoskeletal fraction.

4.6 Quantitative RT-PCR

RNA was isolated using the NucleoSpin RNA Plus Mini kit for RNA purification Macherey-Nagel (#740955.25) according to the manufacturer's instructions. Isolated RNA was converted to cDNA by reverse-transcription reaction using random primers, dNTP mix (Thermo Fisher Scientific), and cDNA kit Promega (M3681) M-MLV Reverse Transcriptase, RNase H Minus, Point Mutant Promega (cat#M3681) as per the manufacturer's protocol. Thermo Fisher Scientific's QuantStudio12K Flex Real-Time PCR System was used to conduct the qPCR. As reference genes, GAPDH and ACTB were employed.

Table 2. Primers used for qPCR.

GENE	SEQUENCE (5'-3')
GAPDH-F (HUMAN)	ACCCACTCCTCCACCTTTGA
GAPDH-R (HUMAN)	TTGCTGTAGCCAAATTCGTTGT
FLAG TAG OF RNF-168 PLASMID-F	ACGATGACGATAAAGCCGCCA
RNF-168-R (EXON1)	AGGGACAGCATAAACTCGCCTT
HER4-F (HUMAN)	GTTCAGGATGTGGACGTTGC
HER4-R (HUMAN)	ACACACCGTCCTTGTCAAAGT
PLSCR4-F (HUMAN)	GGGTTTCTCATGGGTCTCTGG
PLSCR4-R (HUMAN)	CCCTATGTGTCTCCAAGCTGC
TGFB2-F (HUMAN)	GCTCTGTGGGTACCTTGATGC
TGFB2-R (HUMAN)	TCCGTTGTTCAGGCACTCTG

4.7 Drug treatments and synergy assays

To determine the effects of the combined treatment of PP2A activators and HDAC inhibitors on the proliferation of NCI-H460 cells, drug synergy assays were used. H460 cells were seeded in 96 well plates at a seeding density of 3000 cells per well. A day after seeding, cells were treated with different concentrations of the drugs, and the synergy score was calculated using the synergy finder tool (Ianevski et al., 2020). To determine the sensitivity of the HDAC inhibitor Panobinostat to PP2A inhibition, H460 cells were seeded in 96 well plates at a seeding density of 3000 cells per well.

A day before seeding, PP2A was inhibited using siRNA-mediated silencing of its subunit, PP2A was inhibited using siRNA-mediated silencing of its subunit, and 24 hours after silencing, cells were treated with different concentrations of Panobinostat. Forty-eight hours before treatment, the cell proliferation was measured using the CellTiter-Glo® Luminescent Cell Viability Assay (#G7571), and the luminescence was read using the BioTek Synergy H1 multi-mode reader. To determine the effect of various epigenetic inhibitors on gene expression, the HCT-116 reporter cells were treated at the IC50 values of various drugs for 48 hours. Cells were then imaged in the Incucyte S3 Live-Cell Analysis System, and the fluorescence intensity was calculated using the ImageJ tool as well as by measuring GFP intensity using western blotting.

Table 3. Drugs used in the study.

DRUGS	TARGET	COMPANY	CATALOGUE NO.
AZACITIDINE	DNMT1	Adooq BioScience	A10105
DECITABINE	DNMT1	SelleckChem	S1200
IBET-151	BET	Adooq BioScience	A11783
JQ1	BET	Adooq BioScience	A12729
MIVEBRESIB	BET	Adooq BioScience	A17226
PANOBINOSTAT	HDAC	Adooq BioScience	A10518
TSA	HDAC	Adooq BioScience	A10947
SP2509	KDM1A	Adooq BioScience	A14443
SELUMETINIB (AZD6244)	MEK	SelleckChem	S1008
DBK-1154	PP2A		
OKADAIC ACID	PP2A	Sigma	O7760
FTY-720	SET	SelleckChem	S5002

4.8 Immunofluorescence staining

To determine the cellular localization of PME-1 and SET proteins immunofluorescence was used. HeLa cells were seeded into the chambered coverslip (80826, Ibidi) at a density of 20,000 cells per well. The subsequent day cell fixation was performed using 4% formaldehyde for 15 minutes at room temperature. Cells were then permeabilized at room temperature for 15 minutes using 1% Triton X-100 in PBS. Cells were further blocked for 30 minutes at RT by adding 10% goat serum diluted in PBS. Cells were then treated with PME-1 and SET antibodies diluted to

1:200 in 10% goat serum and incubated overnight at 4 °C. Cells were then washed thrice in PBS and treated with secondary antibodies (1:1000 in 10% goat serum) for one hour at RT. The nucleus was stained using a 1:2000 concentration of DAPI diluted in PBS and incubated for 15 minutes at RT. Imaging was done using the 3i CSU-W1 spinning disk confocal microscope.

4.9 Statistical analyses

As stated in the figure legends, every experiment was conducted at least three times. Statistical analyses were done using GraphPad Prism (GraphPad Software; www.graphpad.com). The Mann-Whitney U test for significance (RNAi screens, reporter assays) or the two-tailed t test (Western blotting) were used to assess the results. The following criteria were used to determine the statistical significance: $P \geq 0.05$, not significant (ns), $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), and $P < 0.0001$ (****).

5 Results

5.1.1 PPP2A and RAS-regulated phosphorylation sites are present on epigenetic proteins (I)

The oncogenic function of RAS is regulated through PP2A inhibition, while the tumor suppressor PP2A is very well characterized as a negative regulator of the RAS signaling pathway (Hahn et al., 1999, 2002; Rangarajan et al., 2004). To understand the convergence between RAS and PP2A activities in cancer, phosphoproteomics screening was conducted in previous studies (Kauko et al., 2015, 2020b). In these studies, RAS was depleted using the siRNA directed against its three isoforms (H/K/N-RAS) resulting in a complete inhibition of RAS activity. At the same time, PP2A was activated by siRNA-mediated inhibition of its cellular inhibitors CIP2A, PME-1 and SET (Fig. 1B). These studies employed HeLa cells as a model system which do not harbour RAS mutation on the other hand have low PP2A activity through the expression of PP2A inhibitory proteins. To understand the roles of RAS and PP2A in co-regulation of the global phosphoproteomes we first integrated the data from these studies. The RAS and PP2A data showed a significant overlap of over 270 phosphosites across 237 proteins (Fig. 1B).

Gene ontology (GO) based analysis of the commonly regulated phosphosites revealed that RAS and PP2A activities show a high convergence in regulating the epigenetic proteins involved in transcriptional repression (Fig. 1B). Transcriptionally active DNA is unmethylated and consists of acetylated histones. Transcriptional repression signaling initially involves the DNMT1-mediated addition of methylation groups to the DNA followed by the recruitment of repressor complexes such as the NuRD and the SIN3 complex, resulting in the histone deacetylation favoring chromatin compaction (Fig. 2A).

The phosphosites regulated by RAS and PP2A showed a high occurrence on many of these proteins involved in the epigenetic repression pathway (Fig. 2B). To further validate these epigenetic proteins as PP2A substrate, we screened them for the presence of PP2A-B56 subunit binding motif. Recently, PP2A has been shown to interact with its substrates through a conserved binding region LxxIxe motif (Hertz et al., 2016). Most of the epigenetic proteins enriched from the phosphoproteomics data showed the presence of the PP2A binding motif (Fig. 2C).

To further address the roles of the selected PP2A targeted epigenetic proteins, we performed their siRNA mediated depletion in KRAS mutant non-small cell lung cancer (NSCLC) cell lines (Fig. S3B). Inhibiting the RAS/PP2A-regulated epigenetic proteins (CHD3, MLLT3, KDM1A, DNMT1, TRIM28, RNF168, DOT1L, SMARCA4) significantly decreased the viability of the lung cancer cells, indicating their role in promoting oncogenic growth. Overall, this data identified the phosphoregulation of epigenetic proteins as a point of convergence of RAS and PP2A activities.

We used bioinformatics analysis to further understand the phosphosites' functional significance. We found that many of the phosphosites are located on functionally essential regions of the proteins, as the Ser 714 of DNMT1 was found to exist on a DNA binding domain. In contrast, the Ser 481 of RNF168 was located in a region important for interacting with histones (Fig. 3A, B). Using site-directed mutagenesis, we mutated the phosphosite of RNF168 to Alanine and examined its impact on the association of RNF168 with its interacting partners. We found that the phosphomutant of RNF168 shows a decreased interaction with TP53BP1 (Fig. 3C, D). The member of the NuRD repressor complex CHD3 is an ATP-dependent helicase (Gu et al., 2005). The alanine mutants of the PP2A/RAS regulated site on CHD3 resulted in its degradation, indicating the importance of this phosphosite in regulating its protein stability (Fig. 3F). Collectively, the functional analysis of the selective phosphosite revealed their important role in regulating various functions such as protein-protein interaction as well as protein stability.

5.1.2 PPP2A and RAS regulate the chromatin recruitment of HDAC1 and HDAC2 (I)

Phosphoproteomics data revealed various phosphosites on HDACs to be regulated by RAS and PP2A, while the structural analysis of HDAC1 revealed the presence of PP2A B56 binding motif (Fig. 2A, B, C), indicating the regulation of HDAC by RAS/PP2A. We employed co-immunoprecipitation to verify the interaction between the PP2A B56 subunit and HDAC1. GFP-tagged HDAC1 and B56 plasmids were expressed in NCI-H460 cells, and the expressed proteins were immunoprecipitated using the GFP trap system (Fig. S3 A). Western blot analysis of the immunoprecipitated lysates indicated a notable interaction between HDAC1 and B56α subunits of PP2A.

HDACs catalyse the deacetylation of histone tails, hence, their chromatin recruitment is vital for functioning. Next, to address the effect of PP2A or RAS manipulations on chromatin recruitment of HDAC1/2, we used cell fractionation assays. PP2A was first activated by siRNA-mediated depletion of its inhibitory proteins (PME-1 and SET) for 72 hours, and cells were subsequently fractioned to

obtain the nuclear and chromatin fractions. Western blot analysis of the fractions showed significant enrichment of HDAC1/2 in the nucleus as well as their increased recruitment to the chromatin (Fig. 4A, B). We further validated the above findings by using pharmacological activators of PP2A. NCI-H460 cells were subjected to PP2A activation either by treating cells with the small molecule activators of PP2A (DTO-61, DBK) or by FTY-720 mediated inhibition of the cellular repressor of PP2A (SET). These results corroborated the above finding that the activation of PP2A in the NSCLC cell line increases nuclear translocation and further chromatin recruitment of HDAC1 and 2 (Fig. 4C, D). The NSCLC cell line NCI-H460 is mutant for KRAS and show hyperactivated RAS signaling. To address the effect of RAS on cellular translocation of HDAC1/2, we depleted the three RAS isoforms (H/K/N) using siRNA. All the RAS isoforms were targeted to completely inhibit RAS activity and prevent the RAS downstream pathway activation. Inhibition of RAS showed an effect similar to PP2A activation, resulting in an increased nuclear translocation and further chromatin recruitment of HDACs (Fig. 4E, F).

To further confirm the interplay between the RAS and PP2A-mediated chromatin recruitment of HDAC and its role in oncogenesis we generated a lung cancer transformation model. HBEC cells were serially transformed by deleting the tumor suppressor p53, expression of HRAS G12V mutant, and the PP2A inhibitory small T (ST) antigen. The transformation potential of the system was validated using the soft agar assay (Fig. 4G, H). HBEC cells with a p53 deletion or simultaneous expression of the mutant HRAS failed to form colonies on soft agar. In contrast an additional inhibition of PP2A by ST expression was able to transform the cells, as evident from an increased colony growth on soft agar. This observation validated the previous reports that the RAS-mediated human cell transformation requires simultaneous inhibition of the tumor suppressor PP2A (W. Chen et al., 2004; Hahn et al., 1999; Rangarajan et al., 2004). We further examined these serially transformed HBEC cells for cellular localization of HDACs (Fig. 4I, J). Interestingly, the nuclear abundance of HDACs did not show a major change upon serial transformation. However, a significant change was observed in their chromatin recruitment as seen from the cell fractionation assay. Deletion of p53 resulted in an increased chromatin recruitment of HDACs, while an addition of the mutant HRAS abolished the increased chromatin recruitment of HDACs. Further inhibition of PP2A by ST expression stabilized the RAS-induced chromatin recruitment of HDACs. These results indicate a link between the chromatin recruitment of HDACs and cell transformation. Apart from PP2A, ST antigen is also known to inhibit p53 and RB. While we already inhibited p53 in the first stage of transformation the further inhibition of RB by ST would only add up to this transformation effect. Hence in the final stage of transformation the effect of ST expression is widely directed towards PP2A inhibition.

To further determine if the chromatin recruitment of HDACs influences its sensitivity to small molecule inhibition, we used the HDAC inhibitor Panobinostat in combination with either PP2A inhibition or activation. PP2A was inhibited using siRNA against its scaffolding subunit A, and Panobinostat was added 24h after silencing. 48h after Panobinostat treatment we analysed cell proliferation using the CellTiter-Glo® Assay (Promega). We found that PP2A inhibition resulted in resistance of the lung cancer cell to Panobinostat with an increase in the IC50 value of the inhibitor from 17.28 nM to 31.64 nM (Fig. S5 A). The IC50 values of the drug in PP2A inhibited cells was almost doubled compared to the control cells. On the other hand, treating cells in combination with PP2A activator DBK-1154 and HDAC inhibitor Panobinostat resulted in increased cell death with a positive synergy score of 5.48 (Fig. S5B, C). To summarise, these results indicate that the chromatin recruitment of HDACs regulated by RAS and PP2A plays an important role in human cell transformation.

5.1.3 PPP2A and RAS antagonize transcriptional regulation (I)

To address RAS and PP2A synergy on transcriptional and epigenome regulation, we employed a gene reporter system developed in the colon cancer cell line HCT-116. The model consisted of an epigenetically silenced SFRP1 gene (with IRES fused GFP) by methylation of its promoter. Treatment of cells with inhibitors of repressor proteins such as DNA methyltransferase (DNMT1) results in the derepression of the silenced gene and GFP expression (Fig. 5A) (Cui et al., 2014). The response of the reporter to the modulation of epigenetic proteins was validated using pharmacological inhibition of various epigenetic repressor proteins (Fig. 5B). Resulting increase in GFP signal was an indication of transcriptional activation upon the inhibition of the epigenetic repressors. We further tested the impact of RAS and PP2A on gene repression in this reporter system. HCT-116 cells harbour KRAS mutation and hence show a hyperactivated RAS signaling. To inhibit RAS activity, the reporter cells were treated with siRNA against the three RAS isoforms (H/K/NRAS) while PP2A was inhibited by siRNA-mediated depletion of its A subunit or by treating cells with its inhibitor Okadaic acid. PP2A was also activated by silencing its inhibitory protein PME1. After RAS or PP2A manipulations, GFP expression was monitored by imaging the cells in incucyte and further western blotting.

As a positive control, treatment of cells with DNMT1 inhibitor 5-aza-deoxycytidine (DAC) resulted in transcriptional activation and further expression of the SFRP1 gene (GFP expression). Inhibition of PP2A either by siRNA or okadaic acid treatment resulted in increased gene expression, while PP2A activation by

depleting its inhibitory protein PME-1 resulted in further repression (Fig. 5D, E). On the other hand, RAS inhibition resulted in a decrease in GFP expression, an indicator of gene repression, and further highlight the role of RAS as a transcriptional activator (Fig. 5G, H). Inhibiting MEK, the downstream effector of RAS, resulted in a similar phenotype indicating the RAS activity to be mediated through the MEK pathway (Fig. 5I). To further validate the response of RAS/PP2A regulated phosphorylation site on protein function we expressed the wild type and phosphomutant for of RNF168 in the reporter cells. The WT RNF168 resulted in an increased transcriptional activation, while the mutant failed to elicit a similar response, indicating the phosphoregulation of RNF-168 as an essential regulator of its activity (Fig. 5K). The data that PP2A and RAS have opposite roles in regulating gene expression mediated through the phosphorylation of epigenetic proteins.

We used RNA sequencing to further evaluate the roles of RAS and PP2A in global gene regulation. RAS and PP2A were depleted in HeLa cells using siRNAs. Consistent with results obtained using the SFRP-GFP reporter, PP2A inhibition resulted predominantly in global gene activation, while RAS inhibition led predominantly to gene repression (Fig. 6A, B). Notably, gene set enrichment analysis of genes activated by PP2A inhibition showed significant association with the gene signature of KRAS upregulation, providing an additional mechanistic layer on how PP2A can antagonize RAS signaling (Fig. 6C, D). Other significant cancer-associated pathway signatures upregulated upon PP2A inhibition included EMT and G2M checkpoint. PP2A inhibition did not enrich any downregulated signatures, supporting the conclusion that PP2A inhibition conveys its biological effects by gene activation.assess

The GSEA of the RAS transcriptome showed that it regulates gene sets like PP2A. RAS and PP2A activity significantly correlated at the inflammatory response, mitotic spindle, Interferon, complement, IL6 JAK-STAT signaling, androgen response, and G2M checkpoint hallmark signatures. This indicated a significant overlap in the molecular functions of PP2A and RAS and their coordination in regulating various pathways. PP2A inhibition, apart from the upregulation of KRAS signaling genes, exclusively resulted in the regulation of other oncogenic pathways such as EMT, protein secretion, and early and late estrogen response.

Transcription factor analysis of the RNA-seq data using the ChIP-X Enrichment Analysis 3 (ChEA3) (Keenan et al., 2019) showed that PP2A significantly regulates the TEAD1 and YAP1 family of transcription factors (Fig. 6E). The formation of Oncogenic PP2A subunits consisting of striatins has recently been shown to regulate YAP1 activity, leading to cellular transformation (J. W. Kim et al., 2020; Kurppa & Westermarck, 2020). YAP1 has been previously shown to provide resistance against KRAS inhibition and is required for cellular transformation (Shao et al., 2014). This synergy between KRAS and YAP1 activates FOS transcription factors, leading to

epithelial to mesenchymal transition. Our RNA-seq data showed significant enrichment of the target genes of the FOS transcription factor upon RAS silencing (Fig. 6F). The TF analysis indicates a possible coregulation of YAP1 activity by PP2A and RAS synergy in oncogenic transformation.

Gene Ontology (GO) analysis of PP2A-inhibited cells showed an enrichment of the "positive regulation of intracellular signal transduction" term, indicating PP2A inhibition as an essential event for the activation of cellular signaling pathways (Fig. S6). Other significant GO terms indicated the role of PP2A in regulating cellular migration, adhesion, and motility (supplementary figure 1). The most significantly enriched GO biological process upon RAS silencing was "negative regulation of cell proliferation activity," confirming the role of RAS as a repressor (Fig. S6). The RNA-seq data and its further analysis confirm the role of PP2A as a global repressor of transcription while RAS as an activator. The data also confirms that RAS and PP2A activity synergies at various levels mechanistically and coregulate different cellular processes.

5.1.4 PPP2A promotes epigenetic repression mechanisms (I)

Since the phosphoproteome data indicated the role of PP2A in regulating DNMT1 and the reporter assay showed a strong response upon PP2A modulation, we first determined the role of PP2A in regulating DNA methylation. Since the phosphoproteome analysis was indicative of the role of PP2A in regulating DNA methylation, we used RRBS (Reduced representation bisulfite sequencing) to address the role of PP2A in regulating global DNA methylation. The siRNA-mediated inhibition of the A subunit of PP2A resulted in a decrease in global DNA methylation levels, indicating an increased gene expression. Of the total 211 differentially methylated regions, 143 regions showed a decrease in methylation marks (hypomethylated), while 68 showed an increase in methylation (hypermethylated) (Fig. 7A, B). Exons had the lowest occupancy of the differentially regulated methylation marks (7%), whereas introns (36%), intergenic (30%), and promoter (27%), had virtually symmetrical distributions (Fig. S7A).

DNA methylation of the promoters is the most studied and essential in regulating gene expression. On the other hand it has been reported that cancer cells show blocks of hypomethylated regions at the intergenic regions (Heller et al., 2013). Inhibition of PP2A by various mechanisms is common in cancer cells, and resulting hypomethylation at the intergenic region indicates a possible mechanism for cancer progression.

To study the role of differentially regulated pathways in regulating biological functions, we performed the GO enrichment analysis of the methylome using the

Enricher tool (Fig. S7D-G) (Kuleshov et al., 2016). One of the most significantly enriched biological processes included nucleocytoplasmic regulation, indicating the role of PP2A in regulating the movement of molecules between the nucleus and cytoplasm through its diphosphatase activity. This nucleocytoplasmic shuttling activity is significant for the functioning of chromatin-interacting proteins. Based on the gene set enrichment analysis the inhibition of PP2A also showed an increase in Epithelial to Mesenchymal transition, indicating its role as a tumor suppressor. Regulation of the NuRD component members by phosphorylation and transcriptional repression mediated by PP2A, indicated that it might be involved in chromatin remodeling. Hence, we depleted PP2A in HeLa and used Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) to analyze its impact on chromatin regulation. Consistent with PP2A's role as a repressor in previous experiments, ATAC-seq analysis showed that the inhibition of PP2A results in chromatin opening (Fig. 7C & S8C). We compared the PP2A-regulated differentially accessible chromatin areas with those observed in clinical samples. Various regions of the PP2A-regulated differentially accessible chromatin (DAP) were found to correlate with those in the patient samples.

These results provide strong evidence for the role of PP2A as a global transcription repressor. PP2A was found to predominantly promote gene repression, DNA hypermethylation, and chromatin condensation. The phosphoregulation of various epigenetic proteins involved in the above pathways indicates they are an essential mediator of PP2A activity.

5.1.5 Transcriptional regulation by PP2A inhibitors PME-1 and SET (II)

Tumor progression requires the inhibition of PP2A irrespective of the mutation status and cancer type. Since PP2A activity is needed for cell cycle progression a complete inhibition of PP2A is lethal and hence PP2A subunits are rarely mutated. To specifically regulate PP2A activity, cancer cells utilize the expression of PP2A inhibitory proteins as the major mechanism for the downregulation of PP2A (Kauko & Westermarck, 2018). Since PP2A is a multimeric protein composed of various subunits, each specialized to carry out a particular function, cancer cells have evolved to express subunit specific PP2A inhibitory proteins. These proteins provide further specificity in PP2A inhibition through their specificity for cellular localization. For instance, CIP2A inhibits the B56α & B56Y subunits of PP2A and is located in the cytoplasm, while SET and PME-1 are primarily located in the nucleus and target the C subunit (Junttila et al., 2007; Kauko & Westermarck, 2018; J. Lee et al., 1996; J. Lee & Stock, 1993).

Transcriptome of CIP2A has been characterized previously and is associated with various oncogenic signatures, including myc (Niemelä et al., 2012). The transcriptome of neither PME-1 nor SET has been characterized and described previously, and it is also not clear why cells express two different nuclear inhibitors of PP2A. Moreover, both PME-1 and SET have been found to target the same subunit of PP2A, further raising questions about the specificity of their roles in the cell.

We initially validated the intracellular localization of PME-1 and SET using immunofluorescence microscopy in HeLa cells where we had previously characterised the CIP2A regulated transcriptome (Niemelä et al., 2012) (Fig 1A). The microscopy images confirmed the nuclear localization of both PME-1 and SET. However, the two proteins showed clear differences in the localization pattern. SET was located exclusively in the nucleoplasm and the nucleolus in the form of sharp round dots (Fig 1Ab). On the other hand, PME-1 was found to occur both in the nucleus and the cytoplasm (Fig 1Ab).

We further confirmed the presence of SET and PME-1 in different cellular compartments using a cell fractionation assay (Fig 1B). For this, we separated the cytoplasmic, nuclear membrane, nuclear soluble, and chromatin fractions of the cell. Western blot analysis of different fractions showed the presence of SET and PME-1 in the nucleus. Shearing of the chromatin using micrococcal nuclease results in the release of chromatin-bound proteins. Both PME-1 and SET eluted in the chromatin fractions together with histone. This further validated that PME-1 and SET are chromatin-associated proteins. This also confirms our earlier results from phosphoproteomics that PME-1 and SET prevent the dephosphorylation of numerous chromatin-associated proteins involved in epigenetic gene control (Kauko et al., 2020a).

Whether both the PP2A inhibitors associate with chromatin directly or in association with a protein complex could not be determined. SET has been shown to associate with the INHAT complex regulating histone acetylation, while the PME-1 has been shown to immunoprecipitate with various chromatin-binding proteins (Gamble et al., 2005; Pokharel et al., 2015). Immunofluorescence showed PME-1 to be localized strongly with the nuclear membrane, while for SET, the association with the nuclear membrane was not detected at all (Fig. 1A). The high amount of PME-1 detected in the cytoplasmic fractions could be a result of the dissociation of membrane-bound PME-1 upon fractionation and its elution in the cytoplasmic fraction.

To systematically characterize the similarities and differences in the roles of the two nuclear inhibitors of PP2A, we compared their transcriptional signatures using RNA sequencing. We employed siRNA-mediated knockdown of PME-1 or SET in HeLa cells as a research model to methodically define the transcriptomes controlled

by these nuclear PAIPs (supplementary figure 1B). HeLA cells do not harbor RAS mutation, on the other hand they have low PP2A activity due to the expression of PP2A inhibitory proteins. Additionally, PP2A inhibition is required for all cancer types irrespective of the mutation status. Using HeLa as the cell model system enabled us to compare the PME-1 and SET-regulated transcriptome with our previously performed phosphoproteomics analysis of PP2A targets, or the transcriptional targets of CIP2A in this same cell type (Kauko et al., 2020a; Niemelä et al., 2012). Using the log (2) fold change > 0.5/-0.5 and FDR 0.05 criteria in data analysis, siRNA inhibition of PME1 resulted in the upregulation of 77 genes and the downregulation of 33 genes. In contrast, SET depletion resulted in the upregulation of 189 genes and the downregulation of 67 genes (Fig. 1E, F).

To further understand the molecular mechanisms behind the PME-1 and SET-associated transcriptional signature, we used the ChIP-X Enrichment Analysis 3 (ChEA3) program to perform transcription factor binding site enrichment analysis (Tables 1 and 2) (Keenan et al., 2019). ChEA3 then compares the submitted gene sets with its database of gene set library and ranks the upstream TF based on the enrichment score. The genes elevated by PME-1 inhibition were the targets of the TFs FOSL2 and GATA2. The downregulated genes, on the other hand, were enriched for the binding sites of transcription factors TFAP2A, followed by the chromatin insulator CTCF. The TFs enriched for the upregulated genes from SET inhibition were NR3C1 and TCF12, whereas the TFs enriched in the downregulated gene sets were RFRA and HSF1. The GATA family (GATA3 and GATA4) and TEAD4 and JUND were enriched among genes elevated by SET and PME-1 depletion.

These results indicate that even though PME-1 and SET are nuclear inhibitors of PP2A, they do not co-localize in the nucleus and show a distinct pattern of occurrence inside the nucleus. The transcriptome regulated by both proteins is highly diversified, as indicated by its enrichment for the different transcription factors.

5.1.6 PME-1 and SET activities diverge in the regulation of biological pathways and gene sets (II)

Both PME-1 and SET have been positively associated with the upregulation of oncogenic pathways (Di Mambro & Esposito, 2022; Kauko et al., 2020a; Kauko & Westermarck, 2018; Kaur et al., 2016).

The differentially regulated genes as well as transcription factors enriched from them showed high divergence between PME-1 and SET. To investigate if this divergence also occurs on the biological pathways and processes regulated by SET and PME-1 we used the KOBASS tool to the KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis of the SET and PME-1 regulated transcriptome in specific cellular pathways (Bu et al., 2021).

The differentially expressed genes from the PME-1 inhibition enriched for the pathways involved in proteoglycans in cancer, the RAP1 signaling pathway, and MAPK signaling in cancer (Fig 2A). Other significantly regulated pathways included the ones involved in regulating cell migration and adhesion and included ECM-receptor interaction, adherens junction, focal adhesion, and regulation of actin cytoskeleton (Fig. 2B). These pathways are critical in controlling cancer cell invasion and metastasis and their association with the PME-1 regulated transcriptome highlights the importance of PME-1 mediated PP2A inhibition in the regulation of oncogenesis. PME-1 regulated transcriptome was also found to be associated with other major oncogenic pathways, such as the RAS signaling and platinum drug resistance (Fig. 2A). Transcriptional signatures enriched upon siRNA-mediated SET depletion led to a significant enrichment of the NF-Kappa B signaling pathway followed by apoptosis. Other pathways associated with the SET-regulated transcriptome included the ErbB signaling pathway and the p53 signaling pathway (Fig 2C).

Overall, the pathway analysis of the differentially regulated genes upon PME-1 or SET inhibition showed significant divergence. While PME-1 and SET shared a few common regulatory pathways, such as proteoglycans in cancer and platinum drug resistance, their influence on the cell signaling pathways varied, converging with the variance shown by their gene expression profile and transcription factor enrichment.

Until now, the transcriptome, the enriched transcription factors, and the regulated pathways associated with PME-1 and SET have shown high divergence. To further address if the PME-1 and SET-regulated gene signatures are associated with other oncogenic gene signatures and if the PME-1 and SET signatures show similarity in this association, we used gene set enrichment analysis (GSEA). GSEA compares the differentially expressed genes with database for its association with a specific phenotype. Transcriptome regulated by PME-1 downregulation was positively linked with the oncogenic MYC and E2F1 gene sets. This is in line with the known contribution of PP2A inhibition in promoting MYC and E2F1 activities in cancer (Laine et al., 2013b; Myant et al., 2015). Unexpectedly, PME-1 inhibition was found to correlate positively with the KRAS-associated gene signatures. SET inhibition downregulated the G2/M checkpoint gene signatures, indicating that its expression is needed for cancer cell progression to mitosis. The transcriptome downregulated upon SET inhibition is significantly associated with the G2/M checkpoint gene signatures, suggesting that SET is involved in the cell cycle control of cancer cells. The p53 pathway's gene signature was the most significantly upregulated pathway associated with SET depletion, offering another potential mechanistic explanation for the robust anti-proliferative action of SET inhibition in various cancer cells (Kauko & Westermarck, 2018). Gene signatures associated with the downregulated

PME-1 or SET were found to be commonly associated with the downregulated E2F1 pathway gene sets, indicating the convergence of both the pathways in regulation of oncogenic transcription factor E2F1.

Together, the pathway and the gene set enrichment analysis showed that PME-1 and SET regulate unique biological functions in cancer while also confirming their involvement in controlling cancer-relevant transcriptomes.

5.1.7 Regulation of DNA methylation by PME-1 and SET (II)

Both PME-1 and SET have previously been shown to be associated with regulating epigenetic complexes. SET has been found to be associated with complexes regulating the chromatin acetylation, while PME-1 has been shown to regulate the nucleo-cytoplasmic regulation of HDAC4, thereby modulating its activity (Gamble et al., 2005; Kaur et al., 2016). Phosphoproteomics screen upon RAS and PP2A modulation revealed a phosphorylation site regulated by the direct activity of SET, while the inhibition of PP2A was found to regulate global DNA methylation patterns (Kauko et al., 2020a; A et al., 2023). Cell fractionation analysis of PME-1 and SET found them in chromatin-bound fractions (Fig 1B). Also, RNA sequencing showed both PME-1 and SET to regulate the transcription of various genes by mainly promoting gene repression. In our previous study, we also found PME-1 to regulate the gene expression of a reporter system sensitive to DNA methylation changes (A et al., 2023). These results prompted us to look more at the regulation of DNA methylation by PME-1 and SET activity.

To study the effects of PME-1 and SET modulations on the regulation of DNA methylation, we used Reduced Representative Bisulfite Sequencing (RRBS). PME-1 and SET were depleted in HeLa cells using three different siRNAs directed against them (supplementary figure 1B), and 72 hours after silencing, DNA was isolated from the depleted cells and subjected to RRBS analysis. The methylome of the PME-1 depleted cells exhibited a global DNA hypomethylation pattern with 3113 hypomethylated sites, while only 191 sites were found to be hypermethylated (Fig. 4A, 4B). On the other hand, SET inhibition resulted in a minor change in the global DNA methylation. Only 140 differentially methylated regions (DMRs) were detected upon SET inhibition; of those, 87 sites were hypermethylated, whereas 53 were hypomethylated (Fig. 4C, 4D). Changes in global DNA methylation patterns upon SET and PME-1 inhibition indicated a drastic difference in their roles in regulating DNA methylation, with PME-1 showing a robust change in global methylation patterns. At the same time, SET had a minor effect. To determine the DMR, genome was tiled in 500 bp non overlapping regions. A difference in 25% methylation percentage and Q value of 0.01 was used a cutoff to compare the knockdown and control samples. DMR are sites close together or adjacent to each other in this window.

The SET and PME-1 regulated methylation sites also differed in their localization across the genetic elements. For PME-1, the introns and intergenic areas (each with 37% of the DMRs) were the most heavily methylated regions, followed by the promoters (18%) and the exons (7%) (Fig. 4C). SET depletion showed a maximum DMR occupancy at the intron regions (31%) similar to PME-1 but revealed a greater DMR occupancy in the promoter (28%) and the intergenic (24%) regions (Fig. 4F).

We further concentrated on the DNA methyltransferase DNMT1 to better comprehend the mechanism behind the DNA hypomethylation and gene upregulation phenotype upon PME-1 depletion. The principal de novo methyltransferase in mammals, DNMT1, is responsible for preserving the DNA methylation marks throughout DNA replication (Hermann et al., 2004). To investigate if the global DNA methylation pattern regulated by PME-1 is through its regulation of DNMT1, we checked the levels of DNMT1 upon PME-1 inhibition. We depleted PME-1 in HeLa cells using three different siRNAs similar to the PME-1 silencing for the DNA methylation analysis using RRBS. Western blot analysis of the whole cell extract showed a decrease in the levels of DNMT1 upon PME-1 silencing (Fig. 4G), indicating that the loss of DNA methylation upon PME-1 inhibition is through the reduction in the levels of DNMT1.

The RRBS analysis showed that PME-1 and SET played divergent roles in controlling DNA methylation, in line with their varied functions in regulating gene expression. While the results provide the first concrete evidence that PME-1 is a potent DNA methylation regulator, SET appears to affect gene expression through a nuclear mechanism other than the control of DNA methylation.

Although the research above revealed a previously unknown function for PME-1 as a regulator of DNA methylation, PME-1 likely also affects gene expression through other nuclear pathways. To understand the degree of overlap between the PME-1-regulated transcriptome and methylome, we did an integrative analysis of both the data sets. The genes upregulated from the RNA sequencing dataset and those containing the hypomethylated regions from the RRBS data set were compared using the metascape tool (Y. Zhou et al., 2019). The data indicated a considerable overlap in shared functions and biological pathways. However, only a small number of the significantly regulated genes (seven) shared characteristics in both datasets (Fig. 5A). The hypomethylated regions and the overexpressed genes upon PME-1 depletion were found to be involved in the regulation of GTPase activity, protein phosphorylation, cell-cell adhesion, morphogenesis, and regulation of GTPase-mediated signal transduction (Fig. 5B, 5C).

The integrated analysis of the hypermethylated and upregulated genes from RRBS and RNA sequencing, respectively, showed a minor overlap as indicated by a set of seven common genes including included ERBB4, PLSCR4, PARD3B,

TGFB2, FRY, SYNE1, NR3C2. To investigate the correlation between these genes upregulated upon PME-1 inhibition and cancer, we checked their expression profiles in the Cancer Genome Atlas (TCGA) data of lung cancer by using the UALCAN tool (Chandrashekar et al., 2017). The analysis showed elevated levels of PME-1 in both LUAD (Lung adenocarcinoma) and LUSC (Lung squamous cell carcinoma) (Fig. S2). On the other hand, the TCGA datasets of LUAD and LUSC showed decreased levels of all these genes (Fig. S2). We further examined these genes' relationships to PME-1 in LUADA and LUSC and discovered that their expression was negatively correlated to PME-1 (Fig. S2).

We then validated these findings in our HeLa cell models previously used for RNA sequencing and RRBS. Using three different siRNA's, we depleted PME-1, and seventy-two hours after transfection checked the expression of the target genes. The quantitative PCR (qRT-PCR) analysis found these genes to be upregulated upon PME-1 silencing, confirming the RNA sequencing. Since we observed a downregulation of these genes in the TCGA data sets, we decided to validate the expression of the most highly regulated gene (HER4) in non-small cell lung cancer (NSCLC). We depleted PME-1 in three NSCLC cell lines (A549, NCI-H358 & NCI-H460) and validated the expression of HER4 using quantitative PCR. All three cell lines showed a significant upregulation in HER4 expression upon PME-1 downregulation (Fig. 6B).

The PME-1-regulated transcriptome and methylome data show a minor overlap, indicating that the global hypomethylation resulting from PME-1 inhibition does not result in gene expression. It can be because the gene expression requires additional levels of control, such as promoter-enhancer interaction, alternate splicing, etc.

6 Discussion

6.1.1 PPP2A and RAS activities converge in regulating epigenetic proteins (I)

PP2A is a Serine/Threonine phosphatase that acts as a tumor suppressor, while RAS proteins are small GTPases that activate various kinases downstream of their signaling cascade and act as an oncogene. It has been very well established that RAS-mediated human cell transformation requires the simultaneous inhibition of PP2A (Hahn et al., 1999; Yu et al., 2001; Rangarajan et al., 2004; Hahn et al., 2002). However, the mechanism behind this convergence in their activity has never been addressed.

RAS was first shown to transform mouse fibroblasts (NIH3T3 cells) by Weinberg and colleaguses (C. Shih et al., 1979). However the prerception of RAS as an independent driver of oncogenesis failed when the introduction of mutant RAS failed top transform primary fibroblasts. This indicated that the transformation of human cells is a multistep process and requires additional factors. It was later found that the human fibroblasts and epithelial cells could be transformed by the introduction of the mutant HRAS together with the catalytic subunit of the human telomerase reverse transcriptase enzyme (hTERT) and the simian virus large T (LT) and small T (ST) antigens. Tumor suppressor retinoblastoma (RB) and p53 were suppressed by the simian virus LT antigen, whereas PP2A was inhibited by the ST. It was discovered that ST's inhibition of PP2A was necessary for transformation because manipulating the other variables could only immortalise the cells (Hahn et al., 1999, 2002).

The basis of this work is the study by WC Hahn and Weinberg, who by utilizing various cellular transformation models showed that the cell transformation by expressing mutant RAS can only be achieved by simultaneous inhibition of PP2A. However, none of the studies have previously charachterized the overlap between RAS and PP2A regulated phosphorylation targets. To first determine the common phosphorylation targets of PP2A and RAS, we have used HeLa as a model system. In HeLa cells we conducted various phosphoproteomics and transcriptomics experiments to determine the overlap between the roles of RAS and PP2A in various

cellular functions. To further validate the effects seen in HeLa cells we employed the KRAS mutant lung cancer cells.

To systematically characterize the phosphoproteomics targets co-regulated by these proteins, we analyzed the data from previously performed phosphoproteomic screens (Kauko et al., 2015, 2020a). Our results revealed that the activity of RAS and PP2A converge in regulating the phosphorylation of epigenetic protein complexes. Most of the coregulated phosphosites occurred on the protein complexes involved in transcriptional repression signaling. This study uncovers a novel finding that epigenetic complexes contain unique phosphosites that are co-regulated by the activity of a tumor suppressor (PP2A) and an oncogene (RAS). Phosphorylation of some of these epigenetic complexes has been previously shown to be important for their function. Still, the role of RAS is poorly understood while the role of PP2A has never been addressed.

DNMT1 is a major maintenance methyltransferase in humans that copies the methylation pattern to newly synthesized DNA (Hermann et al., 2004). Many phosphorylation sites of DNMT1 have been identified in high throughput experiments, but only a limited number of them have been associated with its enzymatic activity or function. A decade ago, it was identified that the phosphorylation of DNMT1 at Ser 143 by the RAS downstream target kinase AKT1 increases its stability (Estève et al., 2011). Ever since, limited progress has been made in understanding the phosphoregulation of DNMT1, with none of the studies addressing the role of protein phosphatases in regulating DNMT1 has been published. Our data indicates that the phosphorylation of Ser 714 on DNMT1 is to be regulated by both RAS and PP2A (Fig 2). We also found this phosphorylation site to be in a functionally important region of DNMT1 (Fig 3A).

Nucleosome Remodeling and Deacetylase complex (NuRD) complex promotes transcriptional repression (A. Y. Lai & Wade, 2011). The enzymatic activity of the NuRD complex includes the histone deacetylation activity catalysed by HDAC1/HDAC2 and the ATP-dependent nucleosomal remodeling catalysed by CHD3. Phosphoregulation of HDAC1/2 is not very well studied, and only casein kinase 2 has been found to regulate their phosphorylation (Pflum et al., 2001; Tsai & Seto, 2002). The present study found RAS and PP2A-regulated phosphosites on HDAC1 and HDAC2 (Fig 2). We further validated the effect of RAS and PP2A on chromatin recruitment of HDAC1/2 and found them to be co-regulated by RAS and PP2A (Fig 4A). CHD3 consists of over fifty phosphorylation sites, but none have been functionally validated. There are no known kinases or phosphatases regulating those sites. Our data revealed Ser 713 of CHD3 to be important for its stability (Fig 3F).

RNF168 is a ubiquitin ligase that recruits 53BP1 to damaged chromatin and aids in DNA double-strand break repair (Kelliher et al., 2022). Despite its role in

regulating various critical cellular processes not even a single phosphosite on RNF168 has been identified to be functionally relevant. This work is first to address the role of phosphorylation of RNF168 and further describes it to be important in regulating protein-protein interaction. We found the PP2A and RAS coregulated site Ser 481 on RNF168 as an essential regulator of the interaction between RNF168 and TP53-BP1 (Fig 2F).

Using site-directed mutagenesis, we showed that many of the phosphorylation sites identified from mass spectrometry data are functionally important. Unfortunately, we could not further validate these phosphosites because specific antibodies or biological assays were unavailable. To summarize, this study provides a rich resource of various phosphosites co-regulated by RAS and PP2A activity. This study is the first to describe the phosphoregulation of epigenetic proteins by RAS and PP2A activity. We have functionally verified many of these phosphosites and found them functionally relevant in regulating various biological processes such as chromatin interaction, protein stability, and protein-protein interaction.

6.1.2 PPP2A promotes chromatin recruitment of HDAC1/2 while RAS opposes it (I)

Phosphoproteomics analysis provided insight into the regulatory mechanisms shared by RAS and PP2A. Transcriptional repression involves the recruitment of the NuRD and Sin3a repressor complexes to the chromatin, which catalyses the deacetylation of histone tails, facilitating chromatin condensation and repression of transcription. The deacetylase activity of these repressor complexes comes from the histone deacetylase enzymes HDAC1 and HDAC2 (A. Y. Lai & Wade, 2011). HDACs are regulated by various post-translational modifications, including phosphorylation (Adenuga et al., 2009; Pflum et al., 2001).

It was recently reported that the B subunit recognizes its substrates through a highly specific binding motif in its substrates known as the LxxIxE motif (Hertz et al., 2016). Bioinformatic analysis of HDAC1 and 2 showed the presence of the B subunit binding motif near the phosphorylation sites regulated by PP2A. To validate the interaction between the B56α subunit of PP2A and HDAC1, we used immunoprecipitation assays, and this study is the first to find the physical interaction between the B56α subunit of PP2A and HDAC1. Previous work had identified an interaction between HDAC2 and the PP2A-C subunit (S. Yoon et al., 2018).

The PP2A/RAS regulated phosphorylation site on HDAC1 identified from the phosphoproteomics data upon RAS inhibition and PP2A activation was Ser 421 and Ser 423 (Fig. 2B). These phosphorylation sites are located next to the PP2A B56

subunit binding motif and have been previously implicated in regulating the biological activity of HDAC1 (Pflum et al., 2001). Pflum and colleagues reported that the Alanine mutants of these phosphosites in HDAC1 resulted in a decreased complex formation and enzymatic activity of HDAC1 (Pflum et al., 2001). However, they only addressed the association of HDAC1 with the members of the NuRD/Sin3 family of proteins. HDAC1 is also found to be associated with other epigenetic complexes, such as the CoRSET complex with the lysine-specific demethylase 1a (LSD1) (Y. Song et al., 2020). Another study by Galasinski and colleagues addressed the role of HDAC phosphorylation and complex formation (Galasinski et al., 2002). Contradictory to the work by Pflum and colleagues, they found that treating cells with the PP2A inhibitor okadaic acid disrupts the HDAC1 and HDAC2 complex formation. This study by Galasinski and colleagues reciprocates with our finding,, indicating that the PP2A-mediated dephosphorylation is crucial for chromatin recruitment and transcriptional repressor activity of HDAC1 and HDAC2 (Galasinski et al., 2002).

Previous studies on the phosphoregulation of HDAC2 further support our observation that the phosphorylation of HDAC2 prevents its biological activity while PP2A-mediated dephosphorylation must enhance its biological activity. Adenuga and colleagues found that cigarette smoke results in increased phosphorylation of HDAC2 in lung epithelial cells both in vitro and in vivo, resulting in decreased HDAC2 activity and further proteasomal degradation (Adenuga et al., 2009). Similar to cigarette smoke the okadaic acid mediated inhibition of PP2A resulted in a decreased enzymatic activity of HDAC2.

Another important finding from this study is the role of RAS and PP2A in regulating the cellular localization of HDAC1/2. Phosphorylation of HDAC1/2 is important for its complex formation and enzymatic activity. Limited studies have addressed the role of phosphorylation in regulating cellular localization of HDAC1/2, which are mainly considered localized in the nucleus. A study found that the phosphorylation of HDAC1 results in its dissociation from the CoREST corepressor complex and further translocation to the cytoplasm, (Gu et al., 2005) while in another study, okadaic acid treatment showed a decrease in nuclear localization of HDAC2 (Adenuga et al., 2009). The above studies corroborate our finding that an increase in HDAC1/2 nuclear localization was observed upon PP2A activation. Additionally, our study is the first to address the cellular localization of HDAC1/2 regulated by cooperation between a tumor promoter RAS and a tumor suppressor PP2A. We have confirmed our findings by both siRNA-mediated and pharmacological means.

Since chromatin recruitment of HDAC is a prerequisite for its histone deacetylase activity, we hypothesized that the failure in chromatin recruitment should lead to resistance against HDAC inhibitors. We found that preventing the

chromatin recruitment of HDAC1 by PP2A inhibition resulted in resistance against the HDAC1 inhibitor Panobinostat (Fig. S5 A). PP2A activation results in an increased chromatin recruitment of HDAC1 and 2, which synergize with the HDAC1 inhibitor Panobinostat (Fig. S5 B, C). Our results indicate a novel method of targeting HDAC by inducing its chromatin recruitment. We also found that the failure to do so imparts resistance to HDAC inhibition therapy.

Our study has further addressed the role of RAS and PP2A-mediated chromatin recruitment of HDAC1/2 concerning cell transformation. For this, we generated a human cell transformation model by stepwise introduction of various factors needed for human cell transformation, including the RAS and PP2A manipulation. Since HDAC1/2 was responsive to PP2A/RAS manipulations, we investigated the correlation between the RAS/PP2A mediated chromatin recruitment of HDAC1/2 and the different stages of cell transformation. We observed a decrease in the chromatin recruitment of HDAC1/2 upon the expression of oncogenic KRAS. The pattern of HDAC chromatin recruitment in the stepwise transformed HBEC cells indicates an association between the KRAS (activation) and PP2A (inhibition) mediated cellular transformation and its convergence on the epigenetic machinery, which involves HDAC1 and HDAC2 (Fig. 2I, J). The HBEC transformation model employed in our work confirms the opposing roles of RAS and PP2A in regulating the cellular localization of HDAC1/2 during human cell transformation. By addressing the chromatin recruitment of HDAC in our serial transformation model, we propose that the human cell transformation mediated by RAS and PP2A is at least partially mediated through the epigenetic mechanisms, as shown by the chromatin recruitment of HDAC1/2.

A limitation of our study is that we did not investigate the changes in the HDAC1 and HDAC2 complex formation or its enzymatic activity upon RAS/PP2A modulations. On the other hand, contrary to previous studies involving PP2A manipulation, our study has employed a more extensive and precise manipulation of PP2A involving siRNA-mediated depletion of its cellular inhibitory proteins CIP2A, PME-1, and SET. We have also demonstrated the effects of PP2A activation on chromatin recruitment of HDACs using various pharmacological activators of PP2A. However, there are possible drawbacks of using this approach. We have used three different siRNAs for each gene, and the gene expression effects are a cumulative effect of the three independent siRNAs. Also, the off-target effect of siRNAs can vary from one cell line to another. So, more cell lines should have been included to alleviate the cell line-specific effects. Transient transfection also results in cellular toxicity. Overall, for a more precise and robust gene expression profiles, CRISPR -Cas9 mediated knockout of the target genes should have been done.

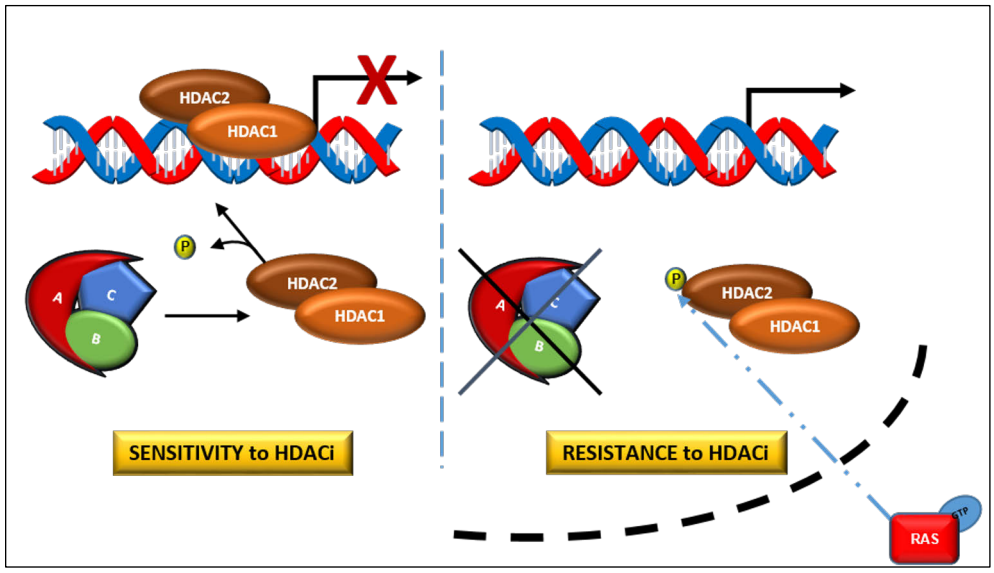

Figure 7. Chromatin recruitment of HDAC1/2 regulated by RAS and PP2A. PP2A dephosphorylates and recruits DACs to the chromatin leading to transcriptional repression while RAS-mediated phosphorylation prevents the chromatin recruitment leading to a constitutively active chromatin.

HDAC is a transcriptional repressor, and the dysregulation of HDAC activity is observed in various cancer types, making it an important target for cancer therapy (Y. Li & Seto, 2016). Our results, as well as previous studies, indicate that phosphorylation of HDAC1 and HDAC2 prevents its nuclear localization and enzymatic activity. We also found that RAS inhibition results in increased chromatin recruitment of HDAC, which should synergise with simultaneous HDAC inhibition. Since simultaneous inhibition of RAS pathway and HDAC has already been tried in various studies, we validated an alternate therapeutic approach involving PP2A activation in combination with HDAC inhibition (M.-W. Chao et al., 2019; Yamada et al., 2018). To summarise, we propose that PP2A-mediated dephosphorylation recruits HDAC1/2 to the chromatin, resulting in transcriptional repression while RAS-mediated phosphorylation prevents it.

6.1.3 PPP2A is a transcriptional repressor while RAS is an activator (I)

To correlate the PP2A/RAS-regulated chromatin recruitment of HDAC1 and HDAC2 with the epigenetic regulation of gene expression, we used a GFP-based reporter system epigenetically silenced by hypermethylation of its promoter. The reporter was sensitive to the modulation of epigenetic factors which were involved

in the regulation of DNA methylation (Fig. 5A). HDACs have been associated with positive regulation of DNA methylation, and various studies have reported DNMT1 to directly interact with HDAC1 and HDAC2 (Rountree et al., 2000; Fuks et al., 2000). It has also been known that HDAC inhibitors result in a reversal of DNA methylation, and similarly, the reporter system employed in our study was sensitive to HDAC inhibitors (Sarkar et al., 2011; Jia et al., 2015).

PP2A inhibition correlated with an increase in gene expression of the reporter system like treating the reporter with the HDAC inhibitor Panobinostat. In contrast, the activation of PP2A by of inhibiting its cellular inhibitors resulted in the suppression of the reporter's gene expression, indicating the role of PP2A as a transcriptional repressor. These observations correlated with our previous finding that PP2A-mediated dephosphorylation recruits HDAC1/2 to the chromatin, which, through their deacetylation activity, represses transcription. RAS prevents this recruitment of HDAC1/2 and thereby leads to transcriptional activation. Whether the PP2A or RAS-mediated phenotype shown by the reporter system is a direct effect of HDAC1/2 mediated chromatin recruitment or through other mechanisms could not be validated in our study. Other possibilities include direct phosphoregulation of epigenetic factors controlling DNA methylation, including the phosphoregulation of DNMT1 or regulation of histone methylation affecting the DNA methylation.

Our study further addressed the impact of RAS and PP2A on global gene expression using RNA sequencing. PP2A primarily led to downregulation of the global gene expression, while RAS showed gene activation. Inhibition of PP2A correlated with the upregulation of KRAS-specific gene signatures indicative of their opposing roles in oncogenesis and transformation (Fig. 6C). PP2A is a negative regulator of the RAS pathways and is known to regulate various downstream targets of RAS through its phosphatase activity (Anderson et al., 1990; Y.-C. Kuo et al., 2008b; Westermarck & Neel, 2020). In this study, RAS and PP2A regulated transcriptome enriched the YAP-TEAD family of transcription factors. Both PP2A and RAS have previously been associated with regulating YAP activity. The RAS pathway was found to promote YAP1 stability and further collude in promoting oncogenic transcription (Hong et al., 2014; Shao et al., 2014). A similar activation of YAP was observed upon the formation of an oncogenic PP2A complex containing striatin subunits (Kurppa & Westermarck, 2020).

Though PP2A and RAS have been implicated in directly regulating YAP, their potential to regulate YAP activity through epigenetic mechanisms has not been explored. Also, the epigenetic regulation of the YAP-TEAD family of transcription factors needs to be extensively characterized. A recent study by Jang and colleagues reported the upregulation of YAP by hypomethylation of its promoter, which was promoted by epigenetic enzymes in response to stiffness of the extracellular matrix (Jang et al., 2021). Modulation of HDAC has been shown to exert varying effects on

YAP and TEAD expression. Treating cells with HDAC inhibitors belinostat and TSA increased TEAD expression while decreasing the phosphorylation and expression of YAP (Basu et al., 2013). Similar findings were reported in another study that showed that the pharmacological inhibition of HDAC downregulates YAP expression as well as inhibits the growth of YAP-driven tumors in xenograft mouse models (Han et al., 2018). Our data strongly supports the activation of oncogenic transcription upon PP2A inhibition. However, we did not address if the enrichment of YAP-TEAD targets is because of a direct effect of PP2A on YAP/TEAD or indirectly through the regulation of epigenetic proteins. There is a strong possibility that PP2A is involved in regulating the activities of the YAP-TEAD family of transcription factors through multiple pathways, which need to be addressed further.

This study also examined the role of PP2A in regulating the major epigenetic processes, such as DNA methylation and chromatin remodeling. In both processes, we found PP2A to act as a repressor, as indicated by an increase in DNA methylation and a decrease in chromatin accessibility. A study by Hervouet and colleagues found that the phosphorylation by AKT disrupts the complex formation of DNMT1 with its interacting partners, resulting in global DNA hypomethylation. This phosphorylation of DNMT1 was associated with transformation and poor prognosis in glioma (Hervouet et al., 2010). Our data showing DNA hypomethylation upon PP2A inhibition orchestrates with this previous finding. We also described a site on DNMT1 coregulated by RAS and PP2A activity (Fig. 3A). Functional characterization of this site will further help in understanding the regulation of DNA methylation by phosphorylation. Moreover, the role of PP2A in regulating the DNA methylation/Chromatin organization has primarily been unexplored.

These results provide the first evidence of the role of PP2A in regulating the core epigenetic processes, including DNA methylation and chromatin organization, and their further association with those in cancer cells. The downstream targets responsible for these phenotypes still need to be explored and validated. Overall, our data strongly suggests PP2A to be a global repressor of gene activation, while RAS is a transcriptional activator.

6.1.4 Nuclear inhibitors of PP2A catalyse highly diversified functions (II)

Expression of various inhibitory proteins (PAIPs) specific to PP2A subunits inhibits the tumor suppressor action of PP2A in cancer cells. The precise functions of these proteins in the transcriptional regulation of gene expression have yet to be charachterized. This is crucial for answering why cancer cells overexpress two distinct nuclear PP2A inhibitors that target the same C subunit. The impacts of CIP2A-regulated gene expression have been well studied (Niemelä et al., 2012),

however, in this study, we focused on characterizing the global transcriptome regulated by PME-1 and SET.

A comparison of the subcellular localization patterns of PME-1 and SET showed striking differences. Immunofluorescence staining showed PME-1 to be associated with nuclear membrane, while SET did not indicate such a pattern (Fig. 1Aa, b). PME-1 has been previously shown to be associated with nuclear lamina, resulting in increased phosphorylation of the associated proteins such as lamins and LAP2A/B (Aakula et al., 2023; Pokharel et al., 2015). PME-1 was also found to occur in the cytoplasm, while SET was localized strictly in the nucleus in the form of sharp round dots (Fig. 1Aa, b). SET has been shown to regulate histone acetylation and transcription by associating with a chromatin-associated INHAT complex (Seo et al., 2001b). Another striking difference between the two PP2A inhibitors was the presence of SET in the nucleolus, where PME-1 was not detected at all. The merged image of the immunofluorescence analysis further indicated that PME-1 and SET show a minor co-localization inside the nucleus, indicating a wide diversity in their functions (Fig. 1Ad).

In the previously conducted phosphoproteomics screen upon PME-1 and SET manipulations, both these PP2A inhibitors were found to influence the phosphorylation of numerous nuclear proteins and the associated signaling pathways such as ribosomal processing, RNA-splicing. In contrast, the CIP2A regulated phosphoproteome was cytoplasmic (Kauko et al., 2020a). To some extent, differences in the cellular localization pattern of the different PP2A inhibitors point towards their different roles in regulating various signaling pathways. The cell fractionation assay further validated the results of immunofluorescence, confirming the additional cytoplasmic localization of PME-1, as seen by its elution in the cytoplasmic fraction (Fig. 1B). On the other hand, SET was located exclusively in the nuclear fractions similar to immunofluorescence staining. The high occurrence of PME-1 in cytoplasmic fractions could be due to its loose association with the nuclear membrane leading to cross-contamination and high amounts of elution in the cytoplasmic fractions. The major inference of the cell fractionation assay was the confirmation of the chromatin recruitment of both PME-1 and SET.

The RNA sequencing results revealed that either PME-1 or SET inhibition resulted in gene upregulation. These PAIPs' repressive effect in gene expression regulation corresponds with our earlier transcriptomics study of the cytoplasmic PP2A inhibitor CIP2A (Niemelä et al., 2012). Further gene set enrichment analysis revealed PME-1 and SET-associated gene signatures highly differ (Fig. 3). According to the findings, PME-1 regulated transcriptome related with the gene signatures associated with the enhanced MYC activity. In contrast, SET positively associated the inhibition of P53 pathway gene signatures, and the upregulation of the G2M checkpoint gene profiles were likewise downregulated by SET inhibition. The

downregulation of p53 function prevents apoptosis and increases mitotic activity, is a critical stage in oncogenic transformation. Based on our findings, SET can coordinate both of these characteristics, offering a mechanistic explanation for SET's high oncogenic activity across many cancer types (Westermarck, 2018). Inhibition of both PME-1 and SET converged at the downregulation of the transcription factor E2F1 gene signatures. The transcription factor E2F1 is linked to oncogenic gene expression and is abundant in cancer. CIP2A has previously been demonstrated to stabilize E2F1 by boosting S364 phosphorylation and promoting E2F1-mediated gene signatures in TNBC (Laine et al., 2013b).

The cellular localization and the gene expression analysis of PME-1 and SET highlight the differences in their biological roles. While PME-1 showed a strong association with nuclear membrane SET was found to strictly reside in the nucleoplasm including the nucleolus. The gene expression data further indicated the PME-1 mediated oncogenic transcription to be associated with MYC while SET with p53.

6.1.5 PME-1 regulates DNA methylation through DNMT1 (II)

DNA methylation is one of the key epigenetic mechanisms dysregulated in cancer (Nishiyama & Nakanishi, 2021). In the previous study, we addressed the role of PP2A in DNA methylation by siRNA-mediated inhibition of the A subunit which was associated with increased DNA methylation (A et al., 2023). Since both PME-1 and SET showed strong nuclear localization and chromatin association and have been previously associated with regulating various nuclear processes, we addressed their impact on DNA methylation (Seo et al., 2001b; Kauko et al., 2020a; Aakula et al., 2023). The global DNA methylome analysis discovered PME-1 to be a major player in regulating the DNA methylation patterns in cancer cells. On the other hand, SET had a minor effect on methylation regulation. Through the activation of oncogenic and repression of tumor suppressor genes, both DNA hypo and hypermethylation play a role in the development of many cancer types (Greger et al., 1989; Kresty et al., 2002; Nishiyama & Nakanishi, 2021).

Another key result from the study was the regulation of DNMT1 protein levels by PME-1. DNA methyltransferases (DNMTs), a class of epigenetic writer proteins that add methyl groups to DNA, process DNA methylation. Inhibition of PME-1 resulted in a decreased stability of DNMT1, explaining the global DNA hypomethylation phenotype observed upon PME-1 inhibition 8Fig. 4G). Various post-translational modifications (PTM) control the function of DNMT1 in cells (Scott et al., 2014). It is known that DNMT1 is controlled by two PTMs that act in opposition to one another: SET7's methylation of Lys142, which lowers the stability of DNMT1, and AKT-mediated phosphorylation of DNMT1 at S143, resulting in

increased stability. Not much research has been done on PP2A's function in controlling DNA methylation. Earlier work in T cells showed that inhibiting the C subunit of PP2A activates the AKT and ERK pathways, stabilizing DNMT1 and increasing DNA methylation. (Sunahori et al., 2013). Our results in the human cancer cell (HeLa) corroborate those findings. However, in the previous study, we found PP2A promotes DNA hypermethylation, which contradicts the present finding that PP2A activation through PME-1 leads to DNA hypomethylation (A et al., 2023). This contradictory result could be due to the difference in the PP2A holoenzyme complex formation upon PME-1 inhibition compared to the previous inhibition of the A subunit.

The analysis of global DNA methylation patterns upon PME-1 or SET inhibition further revealed the differences in gene regulatory mechanisms controlled by both these proteins. Another possible explanation for the difference in DNA methylation pattern is the low activity of PP2A in HeLa cells due to the expression of its inhibitory proteins PME-1 and SET. Hence, activation of PP2A by PME-1 inhibition resulted in a more profound effect compared to the siRNA-mediated PP2A inhibition, which has a feeble effect due to the preexisting suppression of PP2A in HeLa cells.

By further comparing the gene expression and DNA methylation data, we found that PME-1 regulates both ERBB4 (HER4) transcriptome and methylome. HER4 has been previously shown to be regulated by DNA methylation, where the treatment of HER4-negative BY20 cells with DNMT1 inhibitor decitabine resulted in demethylation at specific CpG islands and elevated HER4 expression (Das et al., 2010). This result was consistent with our study, where PME-1 inhibition first led to demethylation of HER4 and subsequent gene expression. PME-1 depletion is also correlated with a decrease in DNMT1 protein levels, indicating the regulation of HER4 to be mediated through DNMT1. However, in the present study, we could not validate if HER4 expression is a direct result of DNMT1 downregulation or is regulated through other pathways. Further validation experiments are needed to confirm this.

To conclude, this work reveals a divergence in the roles of the PP2A inhibitory proteins PME-1 and SET in controlling oncogenic transcription. We have discovered a PME-1-specific function in regulating DNA methylation through the regulation of DNMT1. It is important to note that while PP2A activation primarily suppresses tumor growth, activation of RAS and ERBB4-mediated oncogenic signaling may occur when PME-1 or SET inhibition is used to activate PP2A. On the other hand, the activation of RAS and HER4 in response to PP2A activation suggests that RAS and/or HER4 targeting in combination with PP2A reactivation therapy may improve the therapeutic vulnerability of cancer cells. However, there are certain limitations of the present study. We have used siRNA mediated depletion of PME-1 and SET

which largely effected cell proliferation and had possible off target effects. Furthermore, the off-target effects of the siRNA vary from one cell line to another. Another limitation is that we have used only one cell line as a model system. To generate robust signatures and further validate the present findings, these transcriptional signatures need to be done in additional cell lines. Moreover, to eliminate the effect of cellular toxicity of transient transfection, a CRISPR-Cas9 mediated knockdown of PME-1 and SET would lead to more reliable gene signatures.

7 Summary/Conclusions

My thesis addressed the previously uncharacterized roles of RAS and PP2A cooperation in regulating protein phosphorylation and discovered an important link between the RAS and PP2A-regulated phosphorylation events and their further impact on regulating the control of epigenetic proteins.

Based on various novel findings supported by experimental evidence, the following conclusions can be made from this thesis:

1. Integrating the PP2A and RAS-regulated phosphoproteomics data, it was found that their activities converge on regulating epigenetic proteins. This thesis validated the functional relevance of some of the RAS and PP2A co-regulated phosphorylation sites in regulating the stability and functioning of the epigenetic proteins.

2. The present study discovered the antagonism in RAS and PP2A activity in regulating the cellular localization of HDAC1 and HDAC2. Using a stepwise transformation model, this work found that the cell transformation effects mediated by RAS and PP2A are at least partially mediated through the epigenetic mechanisms, as shown by the chromatin recruitment of HDAC1/2.

3. This study provides the first evidence of the role of PP2A in regulating the core epigenetic processes, including DNA methylation and chromatin organization. Using various epigenetic tools, it was found that PP2A promotes DNA methylation and chromatin condensation.

4. This study found the two nuclear inhibitors of PP2A (PME-1 and SET) to regulate highly diverse functions. While PME-1-mediated oncogenic transcription was found to be associated with MYC, the SET-regulated transcriptional signatures were found to be associated with p53. The present study also discovered a previously unknown role of PME-1 in regulating DNA methylation.

Acknowledgements

This thesis work was carried out at the Faculty of Medicine, Department of Pathology, University of Turku and Turku Bioscience. First, I would like to thank Professor Riitta Lahesmaa, the director of Turku Bioscience, and Professor John Eriksson, the former director, for establishing a cutting-edge facility and an encouraging environment for scientists to pursue their research. I would like to express my appreciation to the entire Turku bioscience community for fostering a collaborative and stimulating research environment. Special thanks go to the technicians and support staff whose dedication ensured the smooth operation of experiments and provided technical assistance whenever needed.

I am profoundly grateful to my supervisor, Professor Jukka Westermarck, for his unwavering support, guidance, and mentorship throughout my thesis. Your mentorship has not only deepened my understanding of the subject matter but has also instilled in me a passion for rigorous research practices. I would also like to acknowledge the countless hours of personal supervision provided by you which greatly contributed to the successful completion of this thesis. I am fortunate to have had the opportunity to work under your leadership.

I am thankful to the members of my research committee, Professor Daniel Abankwa and Associate Professor Riikka Lund for their guidance and support in my research work. Their diverse perspectives have enriched the quality of my work and broadened my scientific horizons. I sincerely thank Associate Professor Chiara Ambrogio and Associate Professor Sakari Vanharanta for reviewing my thesis. Their insightful feedback and constructive criticism have greatly improved this thesis. I would like to express my gratitude to Professor Tilman Brummer, for agreeing to be my opponent. I look forward to a spirited scientific exchange.

I am extremely grateful to the Turku Doctoral Programme of Molecular Medicine (TuDMM), for accepting me as a doctoral candidate in Pathology at the University of Turku Faculty of Medicine. I thank Professor Pekka Taimen, Head of the Pathology Department, Outi Irjala, Chief Academic Officer, and Coordinator Eeva Valve for their administrative support during my PhD studies. I am indebted to the funding agencies, including the Academy of Finland, Ida Montinin säätiön, Jane and Aatos Erkko Foundation, Maud Kuistila Memorial Foundation, Paulon Säätiö,

Turku Doctoral Programme of Molecular Medicine, and Turku University Foundation, for their financial support, which made this research possible.

My heartfelt thanks go to all my collaborators and especially the co-authors who contributed to the research articles included in this thesis: Anna Aakula, Francesco Tabaro, Reetta Nätkin, Jesse Kamila, Henrik Honkanen, Matthieu Schapira, Cheryl Arrowsmith and Matti Nykter. I am also thankful to Saverio Minucci for hosting me in his lab and his entire group for their help and support.

I also extend my appreciation to the core facilities and the staff of the Turku Bioscience Centre for their help and support: Michale Courtney and the screening unit for helping with siRNA screens, Arttu, Pekka, and the entire proteomics core for helping with SRM assays, Ilkka Paatero for zebrafish experiments, Kari and Zsuzsanna from the viral vector core, Jouko and Ketlin from the cell imaging and the entire sequencing and bioinformatics unit.

I would like to express my gratitude to the current and former members of the Westermarck lab, with whom I have had the pleasure of working over the years. Thank you, Tiina, for all your help with paperwork, translations, applications, and organizing group events and for creating a conducive and organized work environment. Taina, thank you for the efficient management of laboratory resources and for helping hand with my experiments. Otto, thank you for introducing me to the project. Your initial guidance was indispensable in completing the work. I sincerely thank my first mentors who familiarised me with the JW lab work ethic: Christian for patiently teaching me soft agar assays, Karolina for cloning and mutagenesis studies, and Aman for her guidance. I am deeply grateful for the friendship, support, and guidance provided by Majid during my PhD. I appreciate the time you have dedicated to answering my questions and offering valuable suggestions. Special thanks to, Srikar, for your friendship. Your willingness to share your knowledge has made a positive difference in my academic and professional journey. I would also like to thank Anna, Jesse, Joel, Henrik, and Maedeh for the scientific teamwork; Laxman for helping with bioinformatic analysis; Oxana for the lively scientific discussions and feedback; Riikka for the in vivo experiments; Joni, Xi, Eleonora, Nikhil, Ram, Priya, Umar, and Phuong for all the fun moments in the lab and their friendship, encouragement, and support. I would also like to acknowledge, Mari, Julia, Nora, Aleksi, Milla, Suvi, and Shruti for their tireless efforts and enthusiasm in the group.

I am deeply grateful to my former lab members from the Abankwa lab: Benoit, Itzi, Tiina, Elina, and Farid for their help and support during my initial days in Turku. I would like to extend my heartfelt gratitude to my master's thesis supervisor Ion Cirstea for introducing me to the world of RAS signaling. Your guidance during my master's thesis laid the foundation for my academic journey and significantly influenced my development as a researcher. I am indebted to my teachers, Joseph

Phillip, Joseph John, and Francis Sir for being a source of inspiration and for their early guidance and encouragement. I am also grateful to all my former colleagues from Ventura Bioscience. Thanks to Srinivas, Siva Ram, Raju, Kalyan, and Arun sir, for your guidance and Deepak, Mirza, and Chinmay for your friendship.

Heartfelt thanks to my friends for their unwavering encouragement, and understanding, during the highs and lows of this academic journey. Thanks, Mitr Manoj, Vivek, Vikas, Deepak, Asad, Atul, Anurag, Gaurav, Ashish, Abhijeet, Christian, Stevan, Abdullah, Mina, Sameer, Jaber, Satya, Marta & Monika for all the shared memories and friendship. I want to extend my heartfelt gratitude to my dear friend Pranshu Sahgal for his continuous guidance, support, and motivation. Thanks to Farid, Saba, Shravani, Sridhar, Neeraj, Meraj, Sana, Moin, Uzma, Afsan, Ankitha, Bilal, Gennadi, Kedar, Deepankar, Nikita, and Prasanna for their friendship and support.

I owe a debt of gratitude to my family, whose unwavering support and belief in my abilities have been a constant source of motivation. Thank you, Papa (Shiwadhar) Maa (Rita), and Bhai (Chandan) for your love, support, and motivation. Finally, to my partner Shampa, your love, encouragement, and sacrifices have been my anchor during this academic journey and sustained me through the challenges of research, writing, and everything in between. I am also deeply grateful to my extended family for their encouragement and understanding.

Turku, January 2024
Mukund Sharma

References

A, A., M, S., F, T., R, N., J, K., H, H., M, S., C, A., M, N., & J, W. (2023). RAS and PP2A activities converge on epigenetic gene regulation. *Life Science Alliance*, *6*(5). https://doi.org/10.26508/lsa.202301928

Aakula, A., Isomursu, A., Rupp, C., Erickson, A., Gupta, N., Kauko, O., Shah, P., Padzik, A., Pokharel, Y. R., Kaur, A., Li, S.-P., Trotman, L., Taimen, P., Rannikko, A., Lammerding, J., Paatero, I., Mirtti, T., Ivaska, J., & Westermarck, J. (2023). PP2A methylesterase PME-1 suppresses anoikis and is associated with therapy relapse of PTEN-deficient prostate cancers. *Molecular Oncology*, *17*(6), 1007–1023. https://doi.org/10.1002/1878-0261.13353

Abraham, D., Podar, K., Pacher, M., Kubicek, M., Welzel, N., Hemmings, B. A., Dilworth, S. M., Mischak, H., Kolch, W., & Baccarini, M. (2000). Raf-1-associated protein phosphatase 2A as a positive regulator of kinase activation. *The Journal of Biological Chemistry*, *275*(29), 22300–22304. https://doi.org/10.1074/jbc.M003259200

Adachi, K., Kohara, T., Nakao, N., Arita, M., Chiba, K., Mishina, T., Sasaki, S., & Fujita, T. (1995). Design, synthesis, and structure-activity relationships of 2-substituted-2-amino-1,3-propanediols: Discovery of a novel immunosuppressant, FTY720. *Bioorganic & Medicinal Chemistry Letters*, *5*(8), 853–856. https://doi.org/10.1016/0960-894X(95)00127-F

Adachi, Y., Pavlakis, G. N., & Copeland, T. D. (1994). Identification and characterization of SET, a nuclear phosphoprotein encoded by the translocation break point in acute undifferentiated leukemia. *The Journal of Biological Chemistry*, *269*(3), 2258–2262.

Adenuga, D., Yao, H., March, T. H., Seagrave, J., & Rahman, I. (2009). Histone deacetylase 2 is phosphorylated, ubiquitinated, and degraded by cigarette smoke. *American Journal of Respiratory Cell and Molecular Biology*, *40*(4), 464–473. https://doi.org/10.1165/rcmb.2008-0255OC

Agger, K., Cloos, P. A. C., Rudkjær, L., Williams, K., Andersen, G., Christensen, J., & Helin, K. (2009). The H3K27me3 demethylase JMJD3 contributes to the activation of the INK4A–ARF locus in response to oncogene- and stress-induced senescence. *Genes & Development*, *23*(10), 1171–1176. https://doi.org/10.1101/gad.510809

Alessi, D. R., Gomez, N., Moorhead, G., Lewis, T., Keyse, S. M., & Cohen, P. (1995). Inactivation of p42 MAP kinase by protein phosphatase 2A and a protein tyrosine phosphatase, but not CL100, in various cell lines. *Current Biology: CB*, *5*(3), 283–295. https://doi.org/10.1016/s0960-9822(95)00059-5

Almeida, L. O., Neto, M. P. C., Sousa, L. O., Tannous, M. A., Curti, C., & Leopoldino, A. M. (2017). SET oncoprotein accumulation regulates transcription through DNA demethylation and histone hypoacetylation. *Oncotarget*, *8*(16), 26802–26818. https://doi.org/10.18632/oncotarget.15818

Al-Yozbaki, M., Jabre, I., Syed, N. H., & Wilson, C. M. (2022). Targeting DNA methyltransferases in non-small-cell lung cancer. *Seminars in Cancer Biology*, *83*, 77–87. https://doi.org/10.1016/j.semcancer.2021.01.005

Anderson, N. G., Maller, J. L., Tonks, N. K., & Sturgill, T. W. (1990). Requirement for integration of signals from two distinct phosphorylation pathways for activation of MAP kinase. *Nature*, *343*(6259), 651–653. https://doi.org/10.1038/343651a0

Ar, C., & Jj, H. (2015). A brief review of nucleosome structure. *FEBS Letters*, *589*(20 Pt A). https://doi.org/10.1016/j.febslet.2015.05.016

Audia, J. E., & Campbell, R. M. (2016). Histone Modifications and Cancer. *Cold Spring Harbor Perspectives in Biology*, *8*(4), a019521. https://doi.org/10.1101/cshperspect.a019521

Azuma, H., Takahara, S., Ichimaru, N., Wang, J. D., Itoh, Y., Otsuki, Y., Morimoto, J., Fukui, R., Hoshiga, M., Ishihara, T., Nonomura, N., Suzuki, S., Okuyama, A., & Katsuoka, Y. (2002). Marked prevention of tumor growth and metastasis by a novel immunosuppressive agent, FTY720, in mouse breast cancer models. *Cancer Research*, *62*(5), 1410–1419.

Bannister, A. J., & Kouzarides, T. (2011). Regulation of chromatin by histone modifications. *Cell Research*, *21*(3), 381–395. https://doi.org/10.1038/cr.2011.22

Barradas, M., Anderton, E., Acosta, J. C., Li, S., Banito, A., Rodriguez-Niedenführ, M., Maertens, G., Banck, M., Zhou, M.-M., Walsh, M. J., Peters, G., & Gil, J. (2009). Histone demethylase JMJD3 contributes to epigenetic control of INK4a/ARF by oncogenic RAS. *Genes & Development*, *23*(10), 1177–1182. https://doi.org/10.1101/gad.511109

Basu, D., Reyes-Múgica, M., & Rebbaa, A. (2013). Histone Acetylation-Mediated Regulation of the Hippo Pathway. *PLoS ONE*, *8*(5), e62478. https://doi.org/10.1371/journal.pone.0062478

Belinsky, S. A., Klinge, D. M., Stidley, C. A., Issa, J.-P., Herman, J. G., March, T. H., & Baylin, S. B. (2003). Inhibition of DNA methylation and histone deacetylation prevents murine lung cancer. *Cancer Research*, *63*(21), 7089–7093.

Bestor, T., Laudano, A., Mattaliano, R., & Ingram, V. (1988). Cloning and sequencing of a cDNA encoding DNA methyltransferase of mouse cells. The carboxyl-terminal domain of the mammalian enzymes is related to bacterial restriction methyltransferases. *Journal of Molecular Biology*, *203*(4), 971–983. https://doi.org/10.1016/0022-2836(88)90122-2

Bird, A., Taggart, M., Frommer, M., Miller, O. J., & Macleod, D. (1985). A fraction of the mouse genome that is derived from islands of nonmethylated, CpG-rich DNA. *Cell*, *40*(1), 91–99. https://doi.org/10.1016/0092-8674(85)90312-5

Bornelöv, S., Reynolds, N., Xenophontos, M., Gharbi, S., Johnstone, E., Floyd, R., Ralser, M., Signolet, J., Loos, R., Dietmann, S., Bertone, P., & Hendrich, B. (2018). The Nucleosome Remodeling and Deacetylation Complex Modulates Chromatin Structure at Sites of Active Transcription to Fine-Tune Gene Expression. *Molecular Cell*, *71*(1), 56-72.e4. https://doi.org/10.1016/j.molcel.2018.06.003

Bostick, M., Kim, J. K., Estève, P.-O., Clark, A., Pradhan, S., & Jacobsen, S. E. (2007). UHRF1 plays a role in maintaining DNA methylation in mammalian cells. *Science (New York, N.Y.)*, *317*(5845), 1760–1764. https://doi.org/10.1126/science.1147939

Bourc'his, D., Xu, G. L., Lin, C. S., Bollman, B., & Bestor, T. H. (2001). Dnmt3L and the establishment of maternal genomic imprints. *Science (New York, N.Y.)*, *294*(5551), 2536–2539. https://doi.org/10.1126/science.1065848

Bracken, A. P., Brien, G. L., & Verrijzer, C. P. (2019). Dangerous liaisons: Interplay between SWI/SNF, NuRD, and Polycomb in chromatin regulation and cancer. *Genes & Development*, *33*(15–16), 936–959. https://doi.org/10.1101/gad.326066.119

Brock, M. V., Hooker, C. M., Ota-Machida, E., Han, Y., Guo, M., Ames, S., Glöckner, S., Piantadosi, S., Gabrielson, E., Pridham, G., Pelosky, K., Belinsky, S. A., Yang, S. C., Baylin, S. B., & Herman, J. G. (2008). DNA Methylation Markers and Early Recurrence in Stage I Lung Cancer. *New England Journal of Medicine*, *358*(11), 1118–1128. https://doi.org/10.1056/NEJMoa0706550

Bu, D., Luo, H., Huo, P., Wang, Z., Zhang, S., He, Z., Wu, Y., Zhao, L., Liu, J., Guo, J., Fang, S., Cao, W., Yi, L., Zhao, Y., & Kong, L. (2021). KOBAS-i: Intelligent prioritization and exploratory visualization of biological functions for gene enrichment analysis. *Nucleic Acids Research*, *49*(W1), W317–W325. https://doi.org/10.1093/nar/gkab447

Buhrman, G., O'Connor, C., Zerbe, B., Kearney, B. M., Napoleon, R., Kovrigina, E. A., Vajda, S., Kozakov, D., Kovrigin, E. L., & Mattos, C. (2011). Analysis of binding site hot spots on the surface of Ras GTPase. *Journal of Molecular Biology*, *413*(4), 773–789. https://doi.org/10.1016/j.jmb.2011.09.011

Cadot, B., Brunetti, M., Coppari, S., Fedeli, S., de Rinaldis, E., Dello Russo, C., Gallinari, P., De Francesco, R., Steinkühler, C., & Filocamo, G. (2009). Loss of histone deacetylase 4 causes segregation defects during mitosis of p53-deficient human tumor cells. *Cancer Research*, *69*(15), 6074–6082. https://doi.org/10.1158/0008-5472.CAN-08-2796

Campbell, J. D., Alexandrov, A., Kim, J., Wala, J., Berger, A. H., Pedamallu, C. S., Shukla, S. A., Guo, G., Brooks, A. N., Murray, B. A., Imielinski, M., Hu, X., Ling, S., Akbani, R., Rosenberg, M., Cibulskis, C., Ramachandran, A., Collisson, E. A., Kwiatkowski, D. J., … Meyerson, M. (2016). Distinct patterns of somatic genome alterations in lung adenocarcinomas and squamous cell carcinomas. *Nature Genetics*, *48*(6), 607–616. https://doi.org/10.1038/ng.3564

Capon, D. J., Seeburg, P. H., McGrath, J. P., Hayflick, J. S., Edman, U., Levinson, A. D., & Goeddel, D. V. (1983). Activation of Ki-ras2 gene in human colon and lung carcinomas by two different point mutations. *Nature*, *304*(5926), Article 5926. https://doi.org/10.1038/304507a0

Cervoni, N., Detich, N., Seo, S.-B., Chakravarti, D., & Szyf, M. (2002). The oncoprotein Set/TAF-1beta, an inhibitor of histone acetyltransferase, inhibits active demethylation of DNA, integrating DNA methylation and transcriptional silencing. *The Journal of Biological Chemistry*, *277*(28), 25026–25031. https://doi.org/10.1074/jbc.M202256200

Chandrashekar, D. S., Bashel, B., Balasubramanya, S. A. H., Creighton, C. J., Ponce-Rodriguez, I., Chakravarthi, B. V. S. K., & Varambally, S. (2017). UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia (New York, N.Y.)*, *19*(8), 649–658. https://doi.org/10.1016/j.neo.2017.05.002

Chang, H.-C., Liu, L.-T., & Hung, W.-C. (2004). Involvement of histone deacetylation in ras-induced down-regulation of the metastasis suppressor RECK. *Cellular Signalling*, *16*(6), 675–679. https://doi.org/10.1016/j.cellsig.2003.11.001

Chang, R., Zhang, Y., Zhang, P., & Zhou, Q. (2017). Snail acetylation by histone acetyltransferase p300 in lung cancer. *Thoracic Cancer*, *8*(3), 131–137. https://doi.org/10.1111/1759-7714.12408

Chao, M.-W., Chang, L.-H., Tu, H.-J., Chang, C.-D., Lai, M.-J., Chen, Y.-Y., Liou, J.-P., Teng, C.-M., & Pan, S.-L. (2019). Combination treatment strategy for pancreatic cancer involving the novel HDAC inhibitor MPT0E028 with a MEK inhibitor beyond K-Ras status. *Clinical Epigenetics*, *11*(1), 85. https://doi.org/10.1186/s13148-019-0681-6

Chao, Y. L., & Pecot, C. V. (2021). Targeting Epigenetics in Lung Cancer. *Cold Spring Harbor Perspectives in Medicine*, *11*(6), a038000. https://doi.org/10.1101/cshperspect.a038000

Chen, J., Martin, B. L., & Brautigan, D. L. (1992). Regulation of protein serine-threonine phosphatase type-2A by tyrosine phosphorylation. *Science (New York, N.Y.)*, *257*(5074), 1261–1264. https://doi.org/10.1126/science.1325671

Chen, R., Zhao, W., Fang, C., Yang, X., & Ji, M. (2020). Histone methyltransferase SETD2: A potential tumor suppressor in solid cancers. *Journal of Cancer*, *11*(11), 3349–3356. https://doi.org/10.7150/jca.38391

Chen, W., Arroyo, J. D., Timmons, J. C., Possemato, R., & Hahn, W. C. (2005). Cancer-associated PP2A Aalpha subunits induce functional haploinsufficiency and tumorigenicity. *Cancer Research*, *65*(18), 8183–8192. https://doi.org/10.1158/0008-5472.CAN-05-1103

Chen, W., Possemato, R., Campbell, K. T., Plattner, C. A., Pallas, D. C., & Hahn, W. C. (2004). Identification of specific PP2A complexes involved in human cell transformation. *Cancer Cell*, *5*(2), 127–136. https://doi.org/10.1016/s1535-6108(04)00026-1

Chiang, C. W., Harris, G., Ellig, C., Masters, S. C., Subramanian, R., Shenolikar, S., Wadzinski, B. E., & Yang, E. (2001). Protein phosphatase 2A activates the proapoptotic function of BAD in interleukin- 3-dependent lymphoid cells by a mechanism requiring 14-3-3 dissociation. *Blood*, *97*(5), 1289–1297. https://doi.org/10.1182/blood.v97.5.1289

Cho, U. S., & Xu, W. (2007). Crystal structure of a protein phosphatase 2A heterotrimeric holoenzyme. *Nature*, *445*(7123), 53–57. https://doi.org/10.1038/nature05351

Choi, Y. L., Soda, M., Yamashita, Y., Ueno, T., Takashima, J., Nakajima, T., Yatabe, Y., Takeuchi, K., Hamada, T., Haruta, H., Ishikawa, Y., Kimura, H., Mitsudomi, T., Tanio, Y., Mano, H., &

ALK Lung Cancer Study Group. (2010). EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. *The New England Journal of Medicine*, *363*(18), 1734–1739. https://doi.org/10.1056/NEJMoa1007478

Clapier, C. R., Iwasa, J., Cairns, B. R., & Peterson, C. L. (2017). Mechanisms of action and regulation of ATP-dependent chromatin-remodelling complexes. *Nature Reviews. Molecular Cell Biology*, *18*(7), 407–422. https://doi.org/10.1038/nrm.2017.26

Coffin, J. M., Varmus, H. E., Bishop, J. M., Essex, M., Hardy, W. D., Martin, G. S., Rosenberg, N. E., Scolnick, E. M., Weinberg, R. A., & Vogt, P. K. (1981). Proposal for naming host cell-derived inserts in retrovirus genomes. *Journal of Virology*, *40*(3), 953–957. https://doi.org/10.1128/JVI.40.3.953-957.1981

Cox, A. D., & Der, C. J. (2010). Ras history. *Small GTPases*, *1*(1), 2–27. https://doi.org/10.4161/sgtp.1.1.12178

Cox, A. D., Fesik, S. W., Kimmelman, A. C., Luo, J., & Der, C. J. (2014). Drugging the undruggable Ras: Mission possible? *Nature Reviews. Drug Discovery*, *13*(11), 828–851. https://doi.org/10.1038/nrd4389

Cui, Y., Hausheer, F., Beaty, R., Zahnow, C., Issa, J. P., Bunz, F., & Baylin, S. B. (2014). A Recombinant Reporter System for Monitoring Reactivation of an Endogenously DNA Hypermethylated Gene. *Cancer Research*, *74*(14), 3834–3843. https://doi.org/10.1158/0008-5472.CAN-13-2287

Das, P. M., Thor, A. D., Edgerton, S. M., Barry, S. K., Chen, D. F., & Jones, F. E. (2010). Reactivation of epigenetically silenced HER4/ERBB4 results in apoptosis of breast tumor cells. *Oncogene*, *29*(37), 5214–5219. https://doi.org/10.1038/onc.2010.271

De Baere, I., Derua, R., Janssens, V., Van Hoof, C., Waelkens, E., Merlevede, W., & Goris, J. (1999). Purification of porcine brain protein phosphatase 2A leucine carboxyl methyltransferase and cloning of the human homologue. *Biochemistry*, *38*(50), 16539–16547. https://doi.org/10.1021/bi991646a

De Palma, R. M., Parnham, S. R., Li, Y., Oaks, J. J., Peterson, Y. K., Szulc, Z. M., Roth, B. M., Xing, Y., & Ogretmen, B. (2019). The NMR-based characterization of the FTY720-SET complex reveals an alternative mechanism for the attenuation of the inhibitory SET-PP2A interaction. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, *33*(6), 7647–7666. https://doi.org/10.1096/fj.201802264R

Deaton, A. M., & Bird, A. (2011). CpG islands and the regulation of transcription. *Genes & Development*, *25*(10), 1010–1022. https://doi.org/10.1101/gad.2037511

DeFeo, D., Gonda, M. A., Young, H. A., Chang, E. H., Lowy, D. R., Scolnick, E. M., & Ellis, R. W. (1981). Analysis of two divergent rat genomic clones homologous to the transforming gene of Harvey murine sarcoma virus. *Proceedings of the National Academy of Sciences of the United States of America*, *78*(6), 3328–3332. https://doi.org/10.1073/pnas.78.6.3328

Deichmann, U. (2016). Epigenetics: The origins and evolution of a fashionable topic. *Developmental Biology*, *416*(1), 249–254. https://doi.org/10.1016/j.ydbio.2016.06.005

Dent, P., Jelinek, T., Morrison, D. K., Weber, M. J., & Sturgill, T. W. (1995). Reversal of Raf-1 activation by purified and membrane-associated protein phosphatases. *Science (New York, N.Y.)*, *268*(5219), 1902–1906. https://doi.org/10.1126/science.7604263

Der, C. J., Krontiris, T. G., & Cooper, G. M. (1982). Transforming genes of human bladder and lung carcinoma cell lines are homologous to the ras genes of Harvey and Kirsten sarcoma viruses. *Proceedings of the National Academy of Sciences of the United States of America*, *79*(11), 3637–3640. https://doi.org/10.1073/pnas.79.11.3637

Dhillon, S. (2023). Adagrasib: First Approval. *Drugs*, *83*(3), 275–285. https://doi.org/10.1007/s40265-023-01839-y

Di Mambro, A., & Esposito, M. T. (2022). Thirty years of SET/TAF1β/I2PP2A: From the identification of the biological functions to its implications in cancer and Alzheimer's disease. *Bioscience Reports*, *42*(11), BSR20221280. https://doi.org/10.1042/BSR20221280

Dobrowsky, R. T., Kamibayashi, C., Mumby, M. C., & Hannun, Y. A. (1993). Ceramide activates heterotrimeric protein phosphatase 2A. *The Journal of Biological Chemistry*, *268*(21), 15523–15530.

Du, Z., Song, J., Wang, Y., Zhao, Y., Guda, K., Yang, S., Kao, H.-Y., Xu, Y., Willis, J., Markowitz, S. D., Sedwick, D., Ewing, R. M., & Wang, Z. (2010). DNMT1 stability is regulated by proteins coordinating deubiquitination and acetylation-driven ubiquitination. *Science Signaling*, *3*(146), ra80. https://doi.org/10.1126/scisignal.2001462

Duong, F. H. T., Christen, V., Berke, J. M., Penna, S. H., Moradpour, D., & Heim, M. H. (2005). Upregulation of protein phosphatase 2Ac by hepatitis C virus modulates NS3 helicase activity through inhibition of protein arginine methyltransferase 1. *Journal of Virology*, *79*(24), 15342–15350. https://doi.org/10.1128/JVI.79.24.15342-15350.2005

Duong, F. H. T., Filipowicz, M., Tripodi, M., La Monica, N., & Heim, M. H. (2004). Hepatitis C virus inhibits interferon signaling through up-regulation of protein phosphatase 2A. *Gastroenterology*, *126*(1), 263–277. https://doi.org/10.1053/j.gastro.2003.10.076

Eichner, L. J., Curtis, S. D., Brun, S. N., McGuire, C. K., Gushterova, I., Baumgart, J. T., Trefts, E., Ross, D. S., Rymoff, T. J., & Shaw, R. J. (2023). HDAC3 is critical in tumor development and therapeutic resistance in Kras-mutant non–small cell lung cancer. *Science Advances*, *9*(11), eadd3243. https://doi.org/10.1126/sciadv.add3243

Esteller, M. (2003). Relevance of DNA methylation in the management of cancer. *The Lancet. Oncology*, *4*(6), 351–358. https://doi.org/10.1016/s1470-2045(03)01115-x

Esteller, M., Hamilton, S. R., Burger, P. C., Baylin, S. B., & Herman, J. G. (1999). Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. *Cancer Research*, *59*(4), 793–797.

Esteller, M., Silva, J. M., Dominguez, G., Bonilla, F., Matias-Guiu, X., Lerma, E., Bussaglia, E., Prat, J., Harkes, I. C., Repasky, E. A., Gabrielson, E., Schutte, M., Baylin, S. B., & Herman, J. G. (2000). Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. *Journal of the National Cancer Institute*, *92*(7), 564–569. https://doi.org/10.1093/jnci/92.7.564

Estève, P.-O., Chang, Y., Samaranayake, M., Upadhyay, A. K., Horton, J. R., Feehery, G. R., Cheng, X., & Pradhan, S. (2011). A methylation and phosphorylation switch between an adjacent lysine and serine determines human DNMT1 stability. *Nature Structural & Molecular Biology*, *18*(1), 42–48. https://doi.org/10.1038/nsmb.1939

Farrell, A. S., Allen-Petersen, B., Daniel, C. J., Wang, X., Wang, Z., Rodriguez, S., Impey, S., Oddo, J., Vitek, M. P., Lopez, C., Christensen, D. J., Sheppard, B., & Sears, R. C. (2014). Targeting inhibitors of the tumor suppressor PP2A for the treatment of pancreatic cancer. *Molecular Cancer Research: MCR*, *12*(6), 924–939. https://doi.org/10.1158/1541-7786.MCR-13-0542

Farrington, C. C., Yuan, E., Mazhar, S., Izadmehr, S., Hurst, L., Allen-Petersen, B. L., Janghorban, M., Chung, E., Wolczanski, G., Galsky, M., Sears, R., Sangodkar, J., & Narla, G. (2020). Protein phosphatase 2A activation as a therapeutic strategy for managing MYC-driven cancers. *The Journal of Biological Chemistry*, *295*(3), 757–770. https://doi.org/10.1074/jbc.RA119.011443

Flegg, C. P., Sharma, M., Medina-Palazon, C., Jamieson, C., Galea, M., Brocardo, M. G., Mills, K., & Henderson, B. R. (2010). Nuclear export and centrosome targeting of the protein phosphatase 2A subunit B56alpha: Role of B56alpha in nuclear export of the catalytic subunit. *The Journal of Biological Chemistry*, *285*(24), 18144–18154. https://doi.org/10.1074/jbc.M109.093294

Fowle, H., Zhao, Z., Xu, Q., Wasserman, J. S., Wang, X., Adeyemi, M., Feiser, F., Kurimchak, A. N., Atar, D., McEwan, B. C., Kettenbach, A. N., Page, R., Peti, W., Dunbrack, R. L., & Graña, X. (2021). PP2A/B55α substrate recruitment as defined by the retinoblastoma-related protein p107. *eLife*, *10*, e63181. https://doi.org/10.7554/eLife.63181

Friedlaender, A., Drilon, A., Banna, G. L., Peters, S., & Addeo, A. (2020). The METeoric rise of MET in lung cancer. *Cancer*, *126*(22), 4826–4837. https://doi.org/10.1002/cncr.33159

Fujiki, H., & Suganuma, M. (1993). Tumor promotion by inhibitors of protein phosphatases 1 and 2A: The okadaic acid class of compounds. *Advances in Cancer Research*, *61*, 143–194. https://doi.org/10.1016/s0065-230x(08)60958-6

Fuks, F., Burgers, W. A., Brehm, A., Hughes-Davies, L., & Kouzarides, T. (2000). DNA methyltransferase Dnmt1 associates with histone deacetylase activity. *Nature Genetics*, *24*(1), 88–91. https://doi.org/10.1038/71750

Galasinski, S. C., Resing, K. A., Goodrich, J. A., & Ahn, N. G. (2002). Phosphatase inhibition leads to histone deacetylases 1 and 2 phosphorylation and disruption of corepressor interactions. *The Journal of Biological Chemistry*, *277*(22), 19618–19626. https://doi.org/10.1074/jbc.M201174200

Gamble, M. J., Erdjument-Bromage, H., Tempst, P., Freedman, L. P., & Fisher, R. P. (2005). The histone chaperone TAF-I/SET/INHAT is required for transcription in vitro of chromatin templates. *Molecular and Cellular Biology*, *25*(2), 797–807. https://doi.org/10.1128/MCB.25.2.797-807.2005

Gao, Q., Steine, E. J., Barrasa, M. I., Hockemeyer, D., Pawlak, M., Fu, D., Reddy, S., Bell, G. W., & Jaenisch, R. (2011). Deletion of the de novo DNA methyltransferase Dnmt3a promotes lung tumor progression. *Proceedings of the National Academy of Sciences*, *108*(44), 18061–18066. https://doi.org/10.1073/pnas.1114946108

Gardiner-Garden, M., & Frommer, M. (1987). CpG islands in vertebrate genomes. *Journal of Molecular Biology*, *196*(2), 261–282. https://doi.org/10.1016/0022-2836(87)90689-9

Gideon, P., John, J., Frech, M., Lautwein, A., Clark, R., Scheffler, J. E., & Wittinghofer, A. (1992). Mutational and kinetic analyses of the GTPase-activating protein (GAP)-p21 interaction: The C-terminal domain of GAP is not sufficient for full activity. *Molecular and Cellular Biology*, *12*(5), 2050–2056. https://doi.org/10.1128/mcb.12.5.2050-2056.1992

Gómez, N., & Cohen, P. (1991). Dissection of the protein kinase cascade by which nerve growth factor activates MAP kinases. *Nature*, *353*(6340), 170–173. https://doi.org/10.1038/353170a0

Gowher, H., & Jeltsch, A. (2002). Molecular enzymology of the catalytic domains of the Dnmt3a and Dnmt3b DNA methyltransferases. *The Journal of Biological Chemistry*, *277*(23), 20409–20414. https://doi.org/10.1074/jbc.M202148200

Greer, E. L., & Shi, Y. (2012). Histone methylation: A dynamic mark in health, disease and inheritance. *Nature Reviews. Genetics*, *13*(5), 343–357. https://doi.org/10.1038/nrg3173

Greger, V., Passarge, E., Höpping, W., Messmer, E., & Horsthemke, B. (1989). Epigenetic changes may contribute to the formation and spontaneous regression of retinoblastoma. *Human Genetics*, *83*(2), 155–158. https://doi.org/10.1007/BF00286709

Gu, H., Liang, Y., Mandel, G., & Roizman, B. (2005). Components of the REST/CoREST/histone deacetylase repressor complex are disrupted, modified, and translocated in HSV-1-infected cells. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(21), 7571–7576. https://doi.org/10.1073/pnas.0502658102

Guan, R. J., Fu, Y., Holt, P. R., & Pardee, A. B. (1999). Association of K-ras mutations with p16 methylation in human colon cancer. *Gastroenterology*, *116*(5), 1063–1071. https://doi.org/10.1016/S0016-5085(99)70009-0

Guo, H., & Damuni, Z. (1993). Autophosphorylation-activated protein kinase phosphorylates and inactivates protein phosphatase 2A. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(6), 2500–2504. https://doi.org/10.1073/pnas.90.6.2500

Gutierrez, A., Pan, L., Groen, R. W. J., Baleydier, F., Kentsis, A., Marineau, J., Grebliunaite, R., Kozakewich, E., Reed, C., Pflumio, F., Poglio, S., Uzan, B., Clemons, P., VerPlank, L., An, F., Burbank, J., Norton, S., Tolliday, N., Steen, H., … Aster, J. C. (2014). Phenothiazines induce PP2A-mediated apoptosis in T cell acute lymphoblastic leukemia. *The Journal of Clinical Investigation*, *124*(2), 644–655. https://doi.org/10.1172/JCI65093

Haanen, T. J., O'Connor, C. M., & Narla, G. (2022). Biased holoenzyme assembly of protein phosphatase 2A (PP2A): From cancer to small molecules. *The Journal of Biological Chemistry*, *298*(12), 102656. https://doi.org/10.1016/j.jbc.2022.102656

Haesen, D., Abbasi Asbagh, L., Derua, R., Hubert, A., Schrauwen, S., Hoorne, Y., Amant, F., Waelkens, E., Sablina, A., & Janssens, V. (2016). Recurrent PPP2R1A Mutations in Uterine

Cancer Act through a Dominant-Negative Mechanism to Promote Malignant Cell Growth. *Cancer Research*, *76*(19), 5719–5731. https://doi.org/10.1158/0008-5472.CAN-15-3342

Hahn, W. C., Counter, C. M., Lundberg, A. S., Beijersbergen, R. L., Brooks, M. W., & Weinberg, R. A. (1999). Creation of human tumour cells with defined genetic elements. *Nature*, *400*(6743), 464–468. https://doi.org/10.1038/22780

Hahn, W. C., Dessain, S. K., Brooks, M. W., King, J. E., Elenbaas, B., Sabatini, D. M., DeCaprio, J. A., & Weinberg, R. A. (2002). Enumeration of the Simian Virus 40 Early Region Elements Necessary for Human Cell Transformation. *Molecular and Cellular Biology*, *22*(7), 2111–2123. https://doi.org/10.1128/MCB.22.7.2111-2123.2002

Hall, A., Marshall, C. J., Spurr, N. K., & Weiss, R. A. (1983). Identification of transforming gene in two human sarcoma cell lines as a new member of the ras gene family located on chromosome 1. *Nature*, *303*(5916), 396–400. https://doi.org/10.1038/303396a0

Han, H., Yang, B., Nakaoka, H. J., Yang, J., Zhao, Y., Le Nguyen, K., Bishara, A. T., Mandalia, T. K., & Wang, W. (2018). Hippo signaling dysfunction induces cancer cell addiction to YAP. *Oncogene*, *37*(50), 6414–6424. https://doi.org/10.1038/s41388-018-0419-5

Hanahan, D. (2022). Hallmarks of Cancer: New Dimensions. *Cancer Discovery*, *12*(1), 31–46. https://doi.org/10.1158/2159-8290.CD-21-1059

He, Y.-F., Li, B.-Z., Li, Z., Liu, P., Wang, Y., Tang, Q., Ding, J., Jia, Y., Chen, Z., Li, L., Sun, Y., Li, X., Dai, Q., Song, C.-X., Zhang, K., He, C., & Xu, G.-L. (2011). Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. *Science (New York, N.Y.)*, *333*(6047), 1303–1307. https://doi.org/10.1126/science.1210944

Heller, G., Babinsky, V. N., Ziegler, B., Weinzierl, M., Noll, C., Altenberger, C., Müllauer, L., Dekan, G., Grin, Y., Lang, G., End-Pfützenreuter, A., Steiner, I., Zehetmayer, S., Döme, B., Arns, B.-M., Fong, K. M., Wright, C. M., Yang, I. A., Klepetko, W., … Zöchbauer-Müller, S. (2013). Genome-wide CpG island methylation analyses in non-small cell lung cancer patients. *Carcinogenesis*, *34*(3), 513–521. https://doi.org/10.1093/carcin/bgs363

Hermann, A., Goyal, R., & Jeltsch, A. (2004). The Dnmt1 DNA-(cytosine-C5)-methyltransferase Methylates DNA Processively with High Preference for Hemimethylated Target Sites *. *Journal of Biological Chemistry*, *279*(46), 48350–48359. https://doi.org/10.1074/jbc.M403427200

Hertz, E. P. T., Kruse, T., Davey, N. E., López-Méndez, B., Sigurðsson, J. O., Montoya, G., Olsen, J. V., & Nilsson, J. (2016). A Conserved Motif Provides Binding Specificity to the PP2A-B56 Phosphatase. *Molecular Cell*, *63*(4), 686–695. https://doi.org/10.1016/j.molcel.2016.06.024

Hervouet, E., Lalier, L., Debien, E., Cheray, M., Geairon, A., Rogniaux, H., Loussouarn, D., Martin, S. A., Vallette, F. M., & Cartron, P.-F. (2010). Disruption of Dnmt1/PCNA/UHRF1 Interactions Promotes Tumorigenesis from Human and Mice Glial Cells. *PLOS ONE*, *5*(6), e11333. https://doi.org/10.1371/journal.pone.0011333

Hoang, P. H., & Landi, M. T. (2022). DNA Methylation in Lung Cancer: Mechanisms and Associations with Histological Subtypes, Molecular Alterations, and Major Epidemiological Factors. *Cancers*, *14*(4), 961. https://doi.org/10.3390/cancers14040961

Hobbs, G. A., Der, C. J., & Rossman, K. L. (2016). RAS isoforms and mutations in cancer at a glance. *Journal of Cell Science*, *129*(7), 1287–1292. https://doi.org/10.1242/jcs.182873

Hofer, F., Fields, S., Schneider, C., & Martin, G. S. (1994). Activated Ras interacts with the Ral guanine nucleotide dissociation stimulator. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(23), 11089–11093.

Holdenrieder, S., Stieber, P., von Pawel, J., Raith, H., Nagel, D., Feldmann, K., & Seidel, D. (2004). Circulating Nucleosomes Predict the Response to Chemotherapy in Patients with Advanced Non–Small Cell Lung Cancer. *Clinical Cancer Research*, *10*(18), 5981–5987. https://doi.org/10.1158/1078-0432.CCR-04-0625

Hong, X., Nguyen, H. T., Chen, Q., Zhang, R., Hagman, Z., Voorhoeve, P. M., & Cohen, S. M. (2014). Opposing activities of the Ras and Hippo pathways converge on regulation of YAP protein turnover. *The EMBO Journal*, *33*(21), 2447–2457. https://doi.org/10.15252/embj.201489385

Hotchkiss, R. D. (1948). The quantitative separation of purines, pyrimidines, and nucleosides by paper chromatography. *The Journal of Biological Chemistry*, *175*(1), 315–332.

Hu, Z., Chen, P.-H., Li, W., Douglas, T., Hines, J., Liu, Y., & Crews, C. M. (2023). Targeted Dephosphorylation of Tau by Phosphorylation Targeting Chimeras (PhosTACs) as a Therapeutic Modality. *Journal of the American Chemical Society*. https://doi.org/10.1021/jacs.2c11706

Hulbert, A., Jusue-Torres, I., Stark, A., Chen, C., Rodgers, K., Lee, B., Griffin, C., Yang, A., Huang, P., Wrangle, J., Belinsky, S. A., Wang, T.-H., Yang, S. C., Baylin, S. B., Brock, M. V., & Herman, J. G. (2017). Early Detection of Lung Cancer using DNA Promoter Hypermethylation in Plasma and Sputum. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research*, *23*(8), 1998–2005. https://doi.org/10.1158/1078-0432.CCR-16-1371

Husni, R. E., Shiba-Ishii, A., Iiyama, S., Shiozawa, T., Kim, Y., Nakagawa, T., Sato, T., Kano, J., Minami, Y., & Noguchi, M. (2016). DNMT3a expression pattern and its prognostic value in lung adenocarcinoma. *Lung Cancer (Amsterdam, Netherlands)*, *97*, 59–65. https://doi.org/10.1016/j.lungcan.2016.04.018

Ianevski, A., Giri, A. K., & Aittokallio, T. (2020). SynergyFinder 2.0: Visual analytics of multi-drug combination synergies. *Nucleic Acids Research*, *48*(W1), W488–W493. https://doi.org/10.1093/nar/gkaa216

Ibanez de Caceres, I., Cortes-Sempere, M., Moratilla, C., Machado-Pinilla, R., Rodriguez-Fanjul, V., Manguán-García, C., Cejas, P., López-Ríos, F., Paz-Ares, L., de CastroCarpeño, J., Nistal, M., Belda-Iniesta, C., & Perona, R. (2010). IGFBP-3 hypermethylation-derived deficiency mediates cisplatin resistance in non-small-cell lung cancer. *Oncogene*, *29*(11), Article 11. https://doi.org/10.1038/onc.2009.454

Ichikawa, T., Shanab, O., Nakahata, S., Shimosaki, S., Manachai, N., Ono, M., Iha, H., Shimoda, K., & Morishita, K. (2020). Novel PRMT5-mediated arginine methylations of HSP90A are essential for maintenance of HSP90A function in NDRG2low ATL and various cancer cells. *Biochimica Et Biophysica Acta. Molecular Cell Research*, *1867*(2), 118615. https://doi.org/10.1016/j.bbamcr.2019.118615

Ihle, N. T., Byers, L. A., Kim, E. S., Saintigny, P., Lee, J. J., Blumenschein, G. R., Tsao, A., Liu, S., Larsen, J. E., Wang, J., Diao, L., Coombes, K. R., Chen, L., Zhang, S., Abdelmelek, M. F., Tang, X., Papadimitrakopoulou, V., Minna, J. D., Lippman, S. M., … Powis, G. (2012). Effect of KRAS oncogene substitutions on protein behavior: Implications for signaling and clinical outcome. *Journal of the National Cancer Institute*, *104*(3), 228–239. https://doi.org/10.1093/jnci/djr523

Ito, A., Kataoka, T. R., Watanabe, M., Nishiyama, K., Mazaki, Y., Sabe, H., Kitamura, Y., & Nojima, H. (2000). A truncated isoform of the PP2A B56 subunit promotes cell motility through paxillin phosphorylation. *The EMBO Journal*, *19*(4), 562–571. https://doi.org/10.1093/emboj/19.4.562

J, K., D, K., T, W., T, I., Po, H., B, B., & Y, F. (2015). Phosphorylation-mediated EZH2 inactivation promotes drug resistance in multiple myeloma. *The Journal of Clinical Investigation*, *125*(12). https://doi.org/10.1172/JCI80325

Jambhekar, A., Dhall, A., & Shi, Y. (2019). Roles and regulation of histone methylation in animal development. *Nature Reviews. Molecular Cell Biology*, *20*(10), 625–641. https://doi.org/10.1038/s41580-019-0151-1

Jang, M., An, J., Oh, S. W., Lim, J. Y., Kim, J., Choi, J. K., Cheong, J.-H., & Kim, P. (2021). Matrix stiffness epigenetically regulates the oncogenic activation of the Yes-associated protein in gastric cancer. *Nature Biomedical Engineering*, *5*(1), Article 1. https://doi.org/10.1038/s41551-020-00657-x

Janssens, V., & Goris, J. (2001). Protein phosphatase 2A: A highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. *The Biochemical Journal*, *353*(Pt 3), 417–439. https://doi.org/10.1042/0264-6021:3530417

Jd, M., & Gd, G. (1979). Specific DNA methylation sites in the vicinity of the chicken beta-globin genes. *Nature*, *280*(5721). https://doi.org/10.1038/280419a0

Jeltsch, A., & Jurkowska, R. Z. (2016). Allosteric control of mammalian DNA methyltransferases—A new regulatory paradigm. *Nucleic Acids Research*, *44*(18), 8556–8575. https://doi.org/10.1093/nar/gkw723

Jia, H., Morris, C. D., Williams, R. M., Loring, J. F., & Thomas, E. A. (2015). HDAC inhibition imparts beneficial transgenerational effects in Huntington's disease mice via altered DNA and histone methylation. *Proceedings of the National Academy of Sciences*, *112*(1), E56–E64. https://doi.org/10.1073/pnas.1415195112

Jin, F., Yang, Z., Shao, J., Tao, J., Reißfelder, C., Loges, S., Zhu, L., & Schölch, S. (2023). ARID1A mutations in lung cancer: Biology, prognostic role, and therapeutic implications. *Trends in Molecular Medicine*, *0*(0). https://doi.org/10.1016/j.molmed.2023.04.005

Junttila, M. R., Puustinen, P., Niemelä, M., Ahola, R., Arnold, H., Böttzauw, T., Ala-aho, R., Nielsen, C., Ivaska, J., Taya, Y., Lu, S.-L., Lin, S., Chan, E. K. L., Wang, X.-J., Grènman, R., Kast, J., Kallunki, T., Sears, R., Kähäri, V.-M., & Westermarck, J. (2007). CIP2A inhibits PP2A in human malignancies. *Cell*, *130*(1), 51–62. https://doi.org/10.1016/j.cell.2007.04.044

Karnoub, A. E., & Weinberg, R. A. (2008). Ras oncogenes: Split personalities. *Nature Reviews Molecular Cell Biology*, *9*(7), Article 7. https://doi.org/10.1038/nrm2438

Kastrinsky, D. B., Sangodkar, J., Zaware, N., Izadmehr, S., Dhawan, N. S., Narla, G., & Ohlmeyer, M. (2015). Reengineered tricyclic anti-cancer agents. *Bioorganic & Medicinal Chemistry*, *23*(19), 6528–6534. https://doi.org/10.1016/j.bmc.2015.07.007

Katayama, R., Friboulet, L., Koike, S., Lockerman, E. L., Khan, T. M., Gainor, J. F., Iafrate, A. J., Takeuchi, K., Taiji, M., Okuno, Y., Fujita, N., Engelman, J. A., & Shaw, A. T. (2014). Two novel ALK mutations mediate acquired resistance to the next-generation ALK inhibitor alectinib. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, *20*(22), 5686–5696. https://doi.org/10.1158/1078-0432.CCR-14-1511

Kau, T. R., Schroeder, F., Ramaswamy, S., Wojciechowski, C. L., Zhao, J. J., Roberts, T. M., Clardy, J., Sellers, W. R., & Silver, P. A. (2003). A chemical genetic screen identifies inhibitors of regulated nuclear export of a Forkhead transcription factor in PTEN-deficient tumor cells. *Cancer Cell*, *4*(6), 463–476. https://doi.org/10.1016/s1535-6108(03)00303-9

Kauko, O., Imanishi, S. Y., Kulesskiy, E., Yetukuri, L., Laajala, T. D., Sharma, M., Pavic, K., Aakula, A., Rupp, C., Jumppanen, M., Haapaniemi, P., Ruan, L., Yadav, B., Suni, V., Varila, T., Corthals, G. L., Reimand, J., Wennerberg, K., Aittokallio, T., & Westermarck, J. (2020a). Phosphoproteome and drug-response effects mediated by the three protein phosphatase 2A inhibitor proteins CIP2A, SET, and PME-1. *Journal of Biological Chemistry*, *295*(13), 4194–4211. https://doi.org/10.1074/jbc.RA119.011265

Kauko, O., Imanishi, S. Y., Kulesskiy, E., Yetukuri, L., Laajala, T. D., Sharma, M., Pavic, K., Aakula, A., Rupp, C., Jumppanen, M., Haapaniemi, P., Ruan, L., Yadav, B., Suni, V., Varila, T., Corthals, G. L., Reimand, J., Wennerberg, K., Aittokallio, T., & Westermarck, J. (2020b). Phosphoproteome and drug-response effects mediated by the three protein phosphatase 2A inhibitor proteins CIP2A, SET, and PME-1. *The Journal of Biological Chemistry*, *295*(13), 4194–4211. https://doi.org/10.1074/jbc.RA119.011265

Kauko, O., Laajala, T. D., Jumppanen, M., Hintsanen, P., Suni, V., Haapaniemi, P., Corthals, G., Aittokallio, T., Westermarck, J., & Imanishi, S. Y. (2015). Label-free quantitative phosphoproteomics with novel pairwise abundance normalization reveals synergistic RAS and CIP2A signaling. *Scientific Reports*, *5*(1), Article 1. https://doi.org/10.1038/srep13099

Kauko, O., O'Connor, C. M., Kulesskiy, E., Sangodkar, J., Aakula, A., Izadmehr, S., Yetukuri, L., Yadav, B., Padzik, A., Laajala, T. D., Haapaniemi, P., Momeny, M., Varila, T., Ohlmeyer, M., Aittokallio, T., Wennerberg, K., Narla, G., & Westermarck, J. (2018). PP2A inhibition is a druggable MEK inhibitor resistance mechanism in KRAS-mutant lung cancer cells. *Science Translational Medicine*, *10*(450), eaaq1093. https://doi.org/10.1126/scitranslmed.aaq1093

Kauko, O., & Westermarck, J. (2018). Non-genomic mechanisms of protein phosphatase 2A (PP2A) regulation in cancer. *The International Journal of Biochemistry & Cell Biology*, *96*, 157–164. https://doi.org/10.1016/j.biocel.2018.01.005

Kaur, A., Denisova, O. V., Qiao, X., Jumppanen, M., Peuhu, E., Ahmed, S. U., Raheem, O., Haapasalo, H., Eriksson, J., Chalmers, A. J., Laakkonen, P., & Westermarck, J. (2016). PP2A Inhibitor PME-1 Drives Kinase Inhibitor Resistance in Glioma Cells. *Cancer Research*, *76*(23), 7001–7011. https://doi.org/10.1158/0008-5472.CAN-16-1134

Kaur, A., & Westermarck, J. (2016). Regulation of protein phosphatase 2A (PP2A) tumor suppressor function by PME-1. *Biochemical Society Transactions*, *44*(6), 1683–1693. https://doi.org/10.1042/BST20160161

Keenan, A. B., Torre, D., Lachmann, A., Leong, A. K., Wojciechowicz, M. L., Utti, V., Jagodnik, K. M., Kropiwnicki, E., Wang, Z., & Ma'ayan, A. (2019). ChEA3: Transcription factor enrichment analysis by orthogonal omics integration. *Nucleic Acids Research*, *47*(W1), W212–W224. https://doi.org/10.1093/nar/gkz446

Kelliher, J., Ghosal, G., & Leung, J. W. C. (2022). New answers to the old RIDDLE: RNF168 and the DNA damage response pathway. *The FEBS Journal*, *289*(9), 2467–2480. https://doi.org/10.1111/febs.15857

Kiel, C., Matallanas, D., & Kolch, W. (2021). The Ins and Outs of RAS Effector Complexes. *Biomolecules*, *11*(2), 236. https://doi.org/10.3390/biom11020236

Kim, H., Kwon, Y. M., Kim, J. S., Han, J., Shim, Y. M., Park, J., & Kim, D.-H. (2006). Elevated mRNA levels of DNA methyltransferase-1 as an independent prognostic factor in primary nonsmall cell lung cancer. *Cancer*, *107*(5), 1042–1049. https://doi.org/10.1002/cncr.22087

Kim, J. W., Berrios, C., Kim, M., Schade, A. E., Adelmant, G., Yeerna, H., Damato, E., Iniguez, A. B., Florens, L., Washburn, M. P., Stegmaier, K., Gray, N. S., Tamayo, P., Gjoerup, O., Marto, J. A., DeCaprio, J., & Hahn, W. C. (2020). STRIPAK directs PP2A activity toward MAP4K4 to promote oncogenic transformation of human cells. *eLife*, *9*, e53003. https://doi.org/10.7554/eLife.53003

Kim, J.-Y., Lee, K.-S., Seol, J.-E., Yu, K., Chakravarti, D., & Seo, S.-B. (2012). Inhibition of p53 acetylation by INHAT subunit SET/TAF-Iβ represses p53 activity. *Nucleic Acids Research*, *40*(1), 75–87. https://doi.org/10.1093/nar/gkr614

Kirsten, W. H., & Mayer, L. A. (1967). Morphologic responses to a murine erythroblastosis virus. *Journal of the National Cancer Institute*, *39*(2), 311–335.

Ko, M., Huang, Y., Jankowska, A. M., Pape, U. J., Tahiliani, M., Bandukwala, H. S., An, J., Lamperti, E. D., Koh, K. P., Ganetzky, R., Liu, X. S., Aravind, L., Agarwal, S., Maciejewski, J. P., & Rao, A. (2010). Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. *Nature*, *468*(7325), 839–843. https://doi.org/10.1038/nature09586

Kouzarides, T. (2007). Chromatin modifications and their function. *Cell*, *128*(4), 693–705. https://doi.org/10.1016/j.cell.2007.02.005

Kresty, L. A., Mallery, S. R., Knobloch, T. J., Song, H., Lloyd, M., Casto, B. C., & Weghorst, C. M. (2002). Alterations of p16(INK4a) and p14(ARF) in patients with severe oral epithelial dysplasia. *Cancer Research*, *62*(18), 5295–5300.

Krontiris, T. G., & Cooper, G. M. (1981). Transforming activity of human tumor DNAs. *Proceedings of the National Academy of Sciences of the United States of America*, *78*(2), 1181–1184. https://doi.org/10.1073/pnas.78.2.1181

Kuleshov, M. V., Jones, M. R., Rouillard, A. D., Fernandez, N. F., Duan, Q., Wang, Z., Koplev, S., Jenkins, S. L., Jagodnik, K. M., Lachmann, A., McDermott, M. G., Monteiro, C. D., Gundersen, G. W., & Ma'ayan, A. (2016). Enrichr: A comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Research*, *44*(W1), W90-97. https://doi.org/10.1093/nar/gkw377

Kundu, A., Shelar, S., Ghosh, A. P., Ballestas, M., Kirkman, R., Nam, H., Brinkley, G. J., Karki, S., Mobley, J. A., Bae, S., Varambally, S., & Sudarshan, S. (2020). 14-3-3 proteins protect AMPK-phosphorylated ten-eleven translocation-2 (TET2) from PP2A-mediated dephosphorylation. *The Journal of Biological Chemistry*, *295*(6), 1754–1766. https://doi.org/10.1074/jbc.RA119.011089

Kuo, M. T., Mandel, J. L., & Chambon, P. (1979). DNA methylation: Correlation with DNase I sensitivity of chicken ovalbumin and conalbumin chromatin. *Nucleic Acids Research*, *7*(8), 2105–2113. https://doi.org/10.1093/nar/7.8.2105

Kuo, Y.-C., Huang, K.-Y., Yang, C.-H., Yang, Y.-S., Lee, W.-Y., & Chiang, C.-W. (2008a). Regulation of phosphorylation of Thr-308 of Akt, cell proliferation, and survival by the B55alpha regulatory subunit targeting of the protein phosphatase 2A holoenzyme to Akt. *The Journal of Biological Chemistry*, *283*(4), 1882–1892. https://doi.org/10.1074/jbc.M709585200

Kuo, Y.-C., Huang, K.-Y., Yang, C.-H., Yang, Y.-S., Lee, W.-Y., & Chiang, C.-W. (2008b). Regulation of phosphorylation of Thr-308 of Akt, cell proliferation, and survival by the B55alpha regulatory subunit targeting of the protein phosphatase 2A holoenzyme to Akt. *The Journal of Biological Chemistry*, *283*(4), 1882–1892. https://doi.org/10.1074/jbc.M709585200

Kurppa, K. J., & Westermarck, J. (2020). Good Guy in Bad Company: How STRNs Convert PP2A into an Oncoprotein. *Cancer Cell*, *38*(1), 20–22. https://doi.org/10.1016/j.ccell.2020.06.011

Lai, A. Y., & Wade, P. A. (2011). NuRD: A multi-faceted chromatin remodeling complex in regulating cancer biology. *Nature Reviews. Cancer*, *11*(8), 588–596. https://doi.org/10.1038/nrc3091

Lai, Q., Xu, Y.-H., Chen, Q., Tang, L., Li, A.-G., Zhang, L.-F., Zhang, C.-F., Song, J.-F., & Du, Z.-Z. (2017). The loss-of-function of DNA methyltransferase 1 by siRNA impairs the growth of non-small cell lung cancer with alleviated side effects via reactivation of RASSF1A and APC in vitro and vivo. *Oncotarget*, *8*(35), 59301–59311. https://doi.org/10.18632/oncotarget.19573

Laine, A., Nagelli, S. G., Farrington, C., Butt, U., Cvrljevic, A. N., Vainonen, J. P., Feringa, F. M., Grönroos, T. J., Gautam, P., Khan, S., Sihto, H., Qiao, X., Pavic, K., Connolly, D. C., Kronqvist, P., Elo, L. L., Maurer, J., Wennerberg, K., Medema, R. H., … Westermarck, J. (2021). CIP2A Interacts with TopBP1 and Drives Basal-Like Breast Cancer Tumorigenesis. *Cancer Research*, *81*(16), 4319–4331. https://doi.org/10.1158/0008-5472.CAN-20-3651

Laine, A., Sihto, H., Come, C., Rosenfeldt, M. T., Zwolinska, A., Niemelä, M., Khanna, A., Chan, E. K., Kähäri, V.-M., Kellokumpu-Lehtinen, P.-L., Sansom, O. J., Evan, G. I., Junttila, M. R., Ryan, K. M., Marine, J.-C., Joensuu, H., & Westermarck, J. (2013a). Senescence sensitivity of breast cancer cells is defined by positive feedback loop between CIP2A and E2F1. *Cancer Discovery*, *3*(2), 182–197. https://doi.org/10.1158/2159-8290.CD-12-0292

Laine, A., Sihto, H., Come, C., Rosenfeldt, M. T., Zwolinska, A., Niemelä, M., Khanna, A., Chan, E. K., Kähäri, V.-M., Kellokumpu-Lehtinen, P.-L., Sansom, O. J., Evan, G. I., Junttila, M. R., Ryan, K. M., Marine, J.-C., Joensuu, H., & Westermarck, J. (2013b). Senescence sensitivity of breast cancer cells is defined by positive feedback loop between CIP2A and E2F1. *Cancer Discovery*, *3*(2), 182–197. https://doi.org/10.1158/2159-8290.CD-12-0292

Land, H., Parada, L. F., & Weinberg, R. A. (1983). Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. *Nature*, *304*(5927), 596–602. https://doi.org/10.1038/304596a0

Langevin, S. M., Kratzke, R. A., & Kelsey, K. T. (2015). Epigenetics of Lung Cancer. *Translational Research : The Journal of Laboratory and Clinical Medicine*, *165*(1), 74–90. https://doi.org/10.1016/j.trsl.2014.03.001

Längst, G., & Manelyte, L. (2015). Chromatin Remodelers: From Function to Dysfunction. *Genes*, *6*(2), 299–324. https://doi.org/10.3390/genes6020299

Lao, D.-H., Yusoff, P., Chandramouli, S., Philp, R. J., Fong, C. W., Jackson, R. A., Saw, T. Y., Yu, C. Y., & Guy, G. R. (2007). Direct binding of PP2A to Sprouty2 and phosphorylation changes are a prerequisite for ERK inhibition downstream of fibroblast growth factor receptor stimulation. *The Journal of Biological Chemistry*, *282*(12), 9117–9126. https://doi.org/10.1074/jbc.M607563200

Lee, D. Y., Hayes, J. J., Pruss, D., & Wolffe, A. P. (1993). A positive role for histone acetylation in transcription factor access to nucleosomal DNA. *Cell*, *72*(1), 73–84. https://doi.org/10.1016/0092-8674(93)90051-q

Lee, J., Chen, Y., Tolstykh, T., & Stock, J. (1996). A specific protein carboxyl methylesterase that demethylates phosphoprotein phosphatase 2A in bovine brain. *Proceedings of the National*

Academy of Sciences of the United States of America, *93*(12), 6043–6047. https://doi.org/10.1073/pnas.93.12.6043

Lee, J., & Stock, J. (1993). Protein phosphatase 2A catalytic subunit is methyl-esterified at its carboxyl terminus by a novel methyltransferase. *The Journal of Biological Chemistry*, *268*(26), 19192–19195.

Lei, Y., Liu, L., Zhang, S., Guo, S., Li, X., Wang, J., Su, B., Fang, Y., Chen, X., Ke, H., & Tao, W. (2017). Hdac7 promotes lung tumorigenesis by inhibiting Stat3 activation. *Molecular Cancer*, *16*(1), 170. https://doi.org/10.1186/s12943-017-0736-2

Lemjabbar-Alaoui, H., Hassan, O., Yang, Y.-W., & Buchanan, P. (2015). Lung cancer: Biology and treatment options. *Biochimica et Biophysica Acta*, *1856*(2), 189–210. https://doi.org/10.1016/j.bbcan.2015.08.002

Leonard, D., Huang, W., Izadmehr, S., O'Connor, C. M., Wiredja, D. D., Wang, Z., Zaware, N., Chen, Y., Schlatzer, D. M., Kiselar, J., Vasireddi, N., Schüchner, S., Perl, A. L., Galsky, M. D., Xu, W., Brautigan, D. L., Ogris, E., Taylor, D. J., & Narla, G. (2020). Selective PP2A Enhancement through Biased Heterotrimer Stabilization. *Cell*, *181*(3), 688-701.e16. https://doi.org/10.1016/j.cell.2020.03.038

Leszinski, G., Gezer, U., Siegele, B., Stoetzer, O., & Holdenrieder, S. (2012). Relevance of histone marks H3K9me3 and H4K20me3 in cancer. *Anticancer Research*, *32*(5), 2199–2205.

Letourneux, C., Rocher, G., & Porteu, F. (2006). B56-containing PP2A dephosphorylate ERK and their activity is controlled by the early gene IEX-1 and ERK. *The EMBO Journal*, *25*(4), 727–738. https://doi.org/10.1038/sj.emboj.7600980

Li, H.-H., Cai, X., Shouse, G. P., Piluso, L. G., & Liu, X. (2007). A specific PP2A regulatory subunit, B56gamma, mediates DNA damage-induced dephosphorylation of p53 at Thr55. *The EMBO Journal*, *26*(2), 402–411. https://doi.org/10.1038/sj.emboj.7601519

Li, X.-Y., Wu, J.-Z., Cao, H.-X., Ma, R., Wu, J.-Q., Zhong, Y.-J., & Feng, J.-F. (2013). Blockade of DNA methylation enhances the therapeutic effect of gefitinib in non-small cell lung cancer cells. *Oncology Reports*, *29*(5), 1975–1982. https://doi.org/10.3892/or.2013.2298

Li, Y., Balakrishnan, V. K., Rowse, M., Wu, C.-G., Bravos, A. P., Yadav, V. K., Ivarsson, Y., Strack, S., Novikova, I. V., & Xing, Y. (2022). Coupling to short linear motifs creates versatile PME-1 activities in PP2A holoenzyme demethylation and inhibition. *eLife*, *11*, e79736. https://doi.org/10.7554/eLife.79736

Li, Y., Gong, H., Wang, P., Zhu, Y., Peng, H., Cui, Y., Li, H., Liu, J., & Wang, Z. (2021). The emerging role of ISWI chromatin remodeling complexes in cancer. *Journal of Experimental & Clinical Cancer Research: CR*, *40*(1), 346. https://doi.org/10.1186/s13046-021-02151-x

Li, Y., & Seto, E. (2016). HDACs and HDAC Inhibitors in Cancer Development and Therapy. *Cold Spring Harbor Perspectives in Medicine*, *6*(10), a026831. https://doi.org/10.1101/cshperspect.a026831

Li, Z., Zhao, J., & Tang, Y. (2023). Advances in the role of SWI/SNF complexes in tumours. *Journal of Cellular and Molecular Medicine*, *27*(8), 1023–1031. https://doi.org/10.1111/jcmm.17709

Liang, R., Li, X., Li, W., Zhu, X., & Li, C. (2021). DNA methylation in lung cancer patients: Opening a "window of life" under precision medicine. *Biomedicine & Pharmacotherapy*, *144*, 112202. https://doi.org/10.1016/j.biopha.2021.112202

Liao, Y.-F., Wu, Y.-B., Long, X., Zhu, S.-Q., Jin, C., Xu, J.-J., & Ding, J.-Y. (2016). High level of BRD4 promotes non-small cell lung cancer progression. *Oncotarget*, *7*(8), 9491–9500. https://doi.org/10.18632/oncotarget.7068

Lin, R.-K., Hsu, H.-S., Chang, J.-W., Chen, C.-Y., Chen, J.-T., & Wang, Y.-C. (2007). Alteration of DNA methyltransferases contributes to 5'CpG methylation and poor prognosis in lung cancer. *Lung Cancer (Amsterdam, Netherlands)*, *55*(2), 205–213. https://doi.org/10.1016/j.lungcan.2006.10.022

Lin, R.-K., Wu, C.-Y., Chang, J.-W., Juan, L.-J., Hsu, H.-S., Chen, C.-Y., Lu, Y.-Y., Tang, Y.-A., Yang, Y.-C., Yang, P.-C., & Wang, Y.-C. (2010). Dysregulation of p53/Sp1 control leads to DNA methyltransferase-1 overexpression in lung cancer. *Cancer Research*, *70*(14), 5807–5817. https://doi.org/10.1158/0008-5472.CAN-09-4161

Liu, C., Yang, Q., Zhu, Q., Lu, X., Li, M., Hou, T., Li, Z., Tang, M., Li, Y., Wang, H., Yang, Y., Wang, H., Zhao, Y., Wen, H., Liu, X., Mao, Z., & Zhu, W.-G. (2020). CBP mediated DOT1L acetylation confers DOT1L stability and promotes cancer metastasis. *Theranostics*, *10*(4), 1758–1776. https://doi.org/10.7150/thno.39013

Liu, L.-M., Tang, Q., Hu, X., Zhao, J.-J., Zhang, Y., Ying, G.-G., & Zhang, F. (2021). Arginine Methyltransferase PRMT1 Regulates p53 Activity in Breast Cancer. *Life (Basel, Switzerland)*, *11*(8), 789. https://doi.org/10.3390/life11080789

Liu, Q., Xiong, J., Xu, D., Hao, N., Zhang, Y., Sang, Y., Wang, Z., Zheng, X., Min, J., Diao, H., Raphael, J., Vareki, S. M., Koropatnick, J., & Min, W. (2021). TdIF1-LSD1 Axis Regulates Epithelial—Mesenchymal Transition and Metastasis via Histone Demethylation of E-Cadherin Promoter in Lung Cancer. *International Journal of Molecular Sciences*, *23*(1), 250. https://doi.org/10.3390/ijms23010250

Liu, Y., Wang, D.-L., Chen, S., Zhao, L., & Sun, F.-L. (2012). Oncogene Ras/Phosphatidylinositol 3-Kinase Signaling Targets Histone H3 Acetylation at Lysine 56. *The Journal of Biological Chemistry*, *287*(49), 41469–41480. https://doi.org/10.1074/jbc.M112.367847

Longin, S., Zwaenepoel, K., Louis, J. V., Dilworth, S., Goris, J., & Janssens, V. (2007). Selection of protein phosphatase 2A regulatory subunits is mediated by the C terminus of the catalytic Subunit. *The Journal of Biological Chemistry*, *282*(37), 26971–26980. https://doi.org/10.1074/jbc.M704059200

Lopez-Serra, L., Ballestar, E., Fraga, M. F., Alaminos, M., Setien, F., & Esteller, M. (2006). A profile of methyl-CpG binding domain protein occupancy of hypermethylated promoter CpG islands of tumor suppressor genes in human cancer. *Cancer Research*, *66*(17), 8342–8346. https://doi.org/10.1158/0008-5472.CAN-06-1932

Luo, J., Ostrem, J., Pellini, B., Imbody, D., Stern, Y., Solanki, H. S., Haura, E. B., & Villaruz, L. C. (2022). Overcoming KRAS-Mutant Lung Cancer. *American Society of Clinical Oncology Educational Book*, *42*, 700–710. https://doi.org/10.1200/EDBK_360354

Luo, Y., Jian, W., Stavreva, D., Fu, X., Hager, G., Bungert, J., Huang, S., & Qiu, Y. (2009). Trans-regulation of histone deacetylase activities through acetylation. *The Journal of Biological Chemistry*, *284*(50), 34901–34910. https://doi.org/10.1074/jbc.M109.038356

Lv, T., Yuan, D., Miao, X., Lv, Y., Zhan, P., Shen, X., & Song, Y. (2012). Over-expression of LSD1 promotes proliferation, migration and invasion in non-small cell lung cancer. *PloS One*, *7*(4), e35065. https://doi.org/10.1371/journal.pone.0035065

Ma, Y., Bai, Y., Mao, H., Hong, Q., Yang, D., Zhang, H., Liu, F., Wu, Z., Jin, Q., Zhou, H., Cao, J., Zhao, J., Zhong, X., & Mao, H. (2016). A panel of promoter methylation markers for invasive and noninvasive early detection of NSCLC using a quantum dots-based FRET approach. *Biosensors & Bioelectronics*, *85*, 641–648. https://doi.org/10.1016/j.bios.2016.05.067

MacLeod, A. R., Rouleau, J., & Szyf, M. (1995). Regulation of DNA Methylation by the Ras Signaling Pathway*. *Journal of Biological Chemistry*, *270*(19), 11327–11337. https://doi.org/10.1074/jbc.270.19.11327

Magenta, A., Fasanaro, P., Romani, S., Di Stefano, V., Capogrossi, M. C., & Martelli, F. (2008). Protein phosphatase 2A subunit PR70 interacts with pRb and mediates its dephosphorylation. *Molecular and Cellular Biology*, *28*(2), 873–882. https://doi.org/10.1128/MCB.00480-07

Mandel, J. L., & Chambon, P. (1979). DNA methylation: Organ specific variations in the methylation pattern within and around ovalbumin and other chicken genes. *Nucleic Acids Research*, *7*(8), 2081–2103. https://doi.org/10.1093/nar/7.8.2081

Martin, M., Geudens, I., Bruyr, J., Potente, M., Bleuart, A., Lebrun, M., Simonis, N., Deroanne, C., Twizere, J.-C., Soubeyran, P., Peixoto, P., Mottet, D., Janssens, V., Hofmann, W.-K., Claes, F., Carmeliet, P., Kettmann, R., Gerhardt, H., & Dequiedt, F. (2013). PP2A regulatory subunit Bα controls endothelial contractility and vessel lumen integrity via regulation of HDAC7. *The EMBO Journal*, *32*(18), 2491–2503. https://doi.org/10.1038/emboj.2013.187

Martin, M., Potente, M., Janssens, V., Vertommen, D., Twizere, J.-C., Rider, M. H., Goris, J., Dimmeler, S., Kettmann, R., & Dequiedt, F. (2008). Protein phosphatase 2A controls the activity of histone deacetylase

7 during T cell apoptosis and angiogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(12), 4727–4732. https://doi.org/10.1073/pnas.0708455105

Matsuoka, Y., Nagahara, Y., Ikekita, M., & Shinomiya, T. (2003). A novel immunosuppressive agent FTY720 induced Akt dephosphorylation in leukemia cells. *British Journal of Pharmacology*, *138*(7), 1303–1312. https://doi.org/10.1038/sj.bjp.0705182

McGinty, R. K., & Tan, S. (2015). Nucleosome structure and function. *Chemical Reviews*, *115*(6), 2255–2273. https://doi.org/10.1021/cr500373h

McKinsey, T. A., Zhang, C. L., Lu, J., & Olson, E. N. (2000). Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. *Nature*, *408*(6808), 106–111. https://doi.org/10.1038/35040593

McKinsey, T. A., Zhang, C. L., & Olson, E. N. (2001). Identification of a signal-responsive nuclear export sequence in class II histone deacetylases. *Molecular and Cellular Biology*, *21*(18), 6312–6321. https://doi.org/10.1128/MCB.21.18.6312-6321.2001

Medina, P. P., Romero, O. A., Kohno, T., Montuenga, L. M., Pio, R., Yokota, J., & Sanchez-Cespedes, M. (2008). Frequent BRG1/SMARCA4–inactivating mutations in human lung cancer cell lines. *Human Mutation*, *29*(5), 617–622. https://doi.org/10.1002/humu.20730

Meissner, A., Mikkelsen, T. S., Gu, H., Wernig, M., Hanna, J., Sivachenko, A., Zhang, X., Bernstein, B. E., Nusbaum, C., Jaffe, D. B., Gnirke, A., Jaenisch, R., & Lander, E. S. (2008). Genome-scale DNA methylation maps of pluripotent and differentiated cells. *Nature*, *454*(7205), 766–770. https://doi.org/10.1038/nature07107

Merisaari, J., Denisova, O. V., Doroszko, M., Le Joncour, V., Johansson, P., Leenders, W. P. J., Kastrinsky, D. B., Zaware, N., Narla, G., Laakkonen, P., Nelander, S., Ohlmeyer, M., & Westermarck, J. (2020). Monotherapy efficacy of blood-brain barrier permeable small molecule reactivators of protein phosphatase 2A in glioblastoma. *Brain Communications*, *2*(1), fcaa002. https://doi.org/10.1093/braincomms/fcaa002

Metzger, E., Wissmann, M., Yin, N., Müller, J. M., Schneider, R., Peters, A. H. F. M., Günther, T., Buettner, R., & Schüle, R. (2005). LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. *Nature*, *437*(7057), 436–439. https://doi.org/10.1038/nature04020

Mohammad, H. P., Smitheman, K. N., Kamat, C. D., Soong, D., Federowicz, K. E., Van Aller, G. S., Schneck, J. L., Carson, J. D., Liu, Y., Butticello, M., Bonnette, W. G., Gorman, S. A., Degenhardt, Y., Bai, Y., McCabe, M. T., Pappalardi, M. B., Kasparec, J., Tian, X., McNulty, K. C., … Kruger, R. G. (2015). A DNA Hypomethylation Signature Predicts Antitumor Activity of LSD1 Inhibitors in SCLC. *Cancer Cell*, *28*(1), 57–69. https://doi.org/10.1016/j.ccell.2015.06.002

Momparler, R. L., & Ayoub, J. (2001). Potential of 5-aza-2′-deoxycytidine (Decitabine) a potent inhibitor of DNA methylation for therapy of advanced non-small cell lung cancer. *Lung Cancer*, *34*, 111–115. https://doi.org/10.1016/S0169-5002(01)00397-X

Moodie, S. A., Willumsen, B. M., Weber, M. J., & Wolfman, A. (1993). Complexes of Ras.GTP with Raf-1 and mitogen-activated protein kinase kinase. *Science (New York, N.Y.)*, *260*(5114), 1658–1661. https://doi.org/10.1126/science.8503013

Moore, A. R., Rosenberg, S. C., McCormick, F., & Malek, S. (2020). RAS-targeted therapies: Is the undruggable drugged? *Nature Reviews Drug Discovery*, *19*(8), Article 8. https://doi.org/10.1038/s41573-020-0068-6

Moore, L. D., Le, T., & Fan, G. (2013). DNA methylation and its basic function. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *38*(1), 23–38. https://doi.org/10.1038/npp.2012.112

Mukhopadhyay, A., Saddoughi, S. A., Song, P., Sultan, I., Ponnusamy, S., Senkal, C. E., Snook, C. F., Arnold, H. K., Sears, R. C., Hannun, Y. A., & Ogretmen, B. (2009). Direct interaction between the inhibitor 2 and ceramide via sphingolipid-protein binding is involved in the regulation of protein phosphatase 2A activity and signaling. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, *23*(3), 751–763. https://doi.org/10.1096/fj.08-120550

Murawska, M., & Brehm, A. (2011). CHD chromatin remodelers and the transcription cycle. *Transcription*, *2*(6), 244–253. https://doi.org/10.4161/trns.2.6.17840

Murray, M. J., Shilo, B. Z., Shih, C., Cowing, D., Hsu, H. W., & Weinberg, R. A. (1981). Three different human tumor cell lines contain different oncogenes. *Cell*, *25*(2), 355–361. https://doi.org/10.1016/0092-8674(81)90054-4

Myant, K., Qiao, X., Halonen, T., Come, C., Laine, A., Janghorban, M., Partanen, J. I., Cassidy, J., Ogg, E.-L., Cammareri, P., Laiterä, T., Okkeri, J., Klefström, J., Sears, R. C., Sansom, O. J., & Westermarck, J. (2015). Serine 62-Phosphorylated MYC Associates with Nuclear Lamins and Its Regulation by CIP2A Is Essential for Regenerative Proliferation. *Cell Reports*, *12*(6), 1019–1031. https://doi.org/10.1016/j.celrep.2015.07.003

Nabet, B., Ó Broin, P., Reyes, J. M., Shieh, K., Lin, C. Y., Will, C. M., Popovic, R., Ezponda, T., Bradner, J. E., Golden, A. A., & Licht, J. D. (2015). Deregulation of the Ras-Erk Signaling Axis Modulates the Enhancer Landscape. *Cell Reports*, *12*(8), 1300–1313. https://doi.org/10.1016/j.celrep.2015.06.078

Nair, S. S., & Kumar, R. (2012). Chromatin remodeling in cancer: A gateway to regulate gene transcription. *Molecular Oncology*, *6*(6), 611–619. https://doi.org/10.1016/j.molonc.2012.09.005

Nair, S. S., Li, D.-Q., & Kumar, R. (2013). A core chromatin remodeling factor instructs global chromatin signaling through multivalent reading of nucleosome codes. *Molecular Cell*, *49*(4), 704–718. https://doi.org/10.1016/j.molcel.2012.12.016

Nakajima, E. C., Drezner, N., Li, X., Mishra-Kalyani, P. S., Liu, Y., Zhao, H., Bi, Y., Liu, J., Rahman, A., Wearne, E., Ojofeitimi, I., Hotaki, L. T., Spillman, D., Pazdur, R., Beaver, J. A., & Singh, H. (2022). FDA Approval Summary: Sotorasib for KRAS G12C-Mutated Metastatic NSCLC. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, *28*(8), 1482–1486. https://doi.org/10.1158/1078-0432.CCR-21-3074

Nan, X., Ng, H. H., Johnson, C. A., Laherty, C. D., Turner, B. M., Eisenman, R. N., & Bird, A. (1998). Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature*, *393*(6683), 386–389. https://doi.org/10.1038/30764

Narita, T., Ito, S., Higashijima, Y., Chu, W. K., Neumann, K., Walter, J., Satpathy, S., Liebner, T., Hamilton, W. B., Maskey, E., Prus, G., Shibata, M., Iesmantavicius, V., Brickman, J. M., Anastassiadis, K., Koseki, H., & Choudhary, C. (2021). Enhancers are activated by p300/CBP activity-dependent PIC assembly, RNAPII recruitment, and pause release. *Molecular Cell*, *81*(10), 2166-2182.e6. https://doi.org/10.1016/j.molcel.2021.03.008

Newbold, R. F., & Overell, R. W. (1983). Fibroblast immortality is a prerequisite for transformation by EJ c-Ha-ras oncogene. *Nature*, *304*(5927), 648–651. https://doi.org/10.1038/304648a0

Niemelä, M., Kauko, O., Sihto, H., Mpindi, J.-P., Nicorici, D., Pernilä, P., Kallioniemi, O.-P., Joensuu, H., Hautaniemi, S., & Westermarck, J. (2012). CIP2A signature reveals the MYC dependency of CIP2A-regulated phenotypes and its clinical association with breast cancer subtypes. *Oncogene*, *31*(39), 4266–4278. https://doi.org/10.1038/onc.2011.599

Nishiyama, A., & Nakanishi, M. (2021). Navigating the DNA methylation landscape of cancer. *Trends in Genetics: TIG*, *37*(11), 1012–1027. https://doi.org/10.1016/j.tig.2021.05.002

Nyce, J. W. (1997). Drug-induced DNA hypermethylation: A potential mediator of acquired drug resistance during cancer chemotherapy. *Mutation Research*, *386*(2), 153–161. https://doi.org/10.1016/s1383-5742(96)00051-8

O'Connor, C. M., Leonard, D., Wiredja, D., Avelar, R. A., Wang, Z., Schlatzer, D., Bryson, B., Tokala, E., Taylor, S. E., Upadhyay, A., Sangodkar, J., Gingras, A.-C., Westermarck, J., Xu, W., DiFeo, A., Brautigan, D. L., Haider, S., Jackson, M., & Narla, G. (2020). Inactivation of PP2A by a recurrent mutation drives resistance to MEK inhibitors. *Oncogene*, *39*(3), 703–717. https://doi.org/10.1038/s41388-019-1012-2

Ogris, E., Du, X., Nelson, K. C., Mak, E. K., Yu, X. X., Lane, W. S., & Pallas, D. C. (1999). A protein phosphatase methylesterase (PME-1) is one of several novel proteins stably associating with two inactive mutants of protein phosphatase 2A. *The Journal of Biological Chemistry*, *274*(20), 14382–14391. https://doi.org/10.1074/jbc.274.20.14382

Okano, M., Bell, D. W., Haber, D. A., & Li, E. (1999). DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell*, *99*(3), 247–257. https://doi.org/10.1016/s0092-8674(00)81656-6

Ostrem, J. M., Peters, U., Sos, M. L., Wells, J. A., & Shokat, K. M. (2013). K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. *Nature*, *503*(7477), Article 7477. https://doi.org/10.1038/nature12796

Otani, Y., Sur, H. P., Rachaiah, G., Namagiri, S., Chowdhury, A., Lewis, C. T., Shimizu, T., Gangaplara, A., Wang, X., Vézina, A., Maric, D., Jackson, S., Yan, Y., Zhengping, Z., Ray-Chaudhury, A., Kumar, S., Ballester, L. Y., Chittiboina, P., Yoo, J. Y., … Banasavadi-Siddegowda, Y. K. (2021). Inhibiting protein phosphatase 2A increases the antitumor effect of protein arginine methyltransferase 5 inhibition in models of glioblastoma. *Neuro-Oncology*, *23*(9), 1481–1493. https://doi.org/10.1093/neuonc/noab014

Parada, L. F., Tabin, C. J., Shih, C., & Weinberg, R. A. (1982). Human EJ bladder carcinoma oncogene is homologue of Harvey sarcoma virus ras gene. *Nature*, *297*(5866), 474–478. https://doi.org/10.1038/297474a0

Park, S.-Y., & Kim, J.-S. (2020). A short guide to histone deacetylases including recent progress on class II enzymes. *Experimental & Molecular Medicine*, *52*(2), 204–212. https://doi.org/10.1038/s12276-020-0382-4

Paroni, G., Cernotta, N., Dello Russo, C., Gallinari, P., Pallaoro, M., Foti, C., Talamo, F., Orsatti, L., Steinkühler, C., & Brancolini, C. (2008). PP2A regulates HDAC4 nuclear import. *Molecular Biology of the Cell*, *19*(2), 655–667. https://doi.org/10.1091/mbc.e07-06-0623

Pavic, K., Gupta, N., Omella, J. D., Derua, R., Aakula, A., Huhtaniemi, R., Määttä, J. A., Höfflin, N., Okkeri, J., Wang, Z., Kauko, O., Varjus, R., Honkanen, H., Abankwa, D., Köhn, M., Hytönen, V. P., Xu, W., Nilsson, J., Page, R., … Westermarck, J. (2023). Structural mechanism for inhibition of PP2A-B56α and oncogenicity by CIP2A. *Nature Communications*, *14*(1), 1143. https://doi.org/10.1038/s41467-023-36693-9

Permpongkosol, S., Wang, J.-D., Takahara, S., Matsumiya, K., Nonomura, N., Nishimura, K., Tsujimura, A., Kongkanand, A., & Okuyama, A. (2002). Anticarcinogenic effect of FTY720 in human prostate carcinoma DU145 cells: Modulation of mitogenic signaling, FAK, cell-cycle entry and apoptosis. *International Journal of Cancer*, *98*(2), 167–172. https://doi.org/10.1002/ijc.10178

Perucho, M., Goldfarb, M., Shimizu, K., Lama, C., Fogh, J., & Wigler, M. (1981). Human-tumor-derived cell lines contain common and different transforming genes. *Cell*, *27*(3 Pt 2), 467–476. https://doi.org/10.1016/0092-8674(81)90388-3

Peterson, R. T., Desai, B. N., Hardwick, J. S., & Schreiber, S. L. (1999). Protein phosphatase 2A interacts with the 70-kDa S6 kinase and is activated by inhibition of FKBP12-rapamycinassociated protein. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(8), 4438–4442. https://doi.org/10.1073/pnas.96.8.4438

Pflum, M. K., Tong, J. K., Lane, W. S., & Schreiber, S. L. (2001). Histone deacetylase 1 phosphorylation promotes enzymatic activity and complex formation. *The Journal of Biological Chemistry*, *276*(50), 47733–47741. https://doi.org/10.1074/jbc.M105590200

Planchard, D., Smit, E. F., Groen, H. J. M., Mazieres, J., Besse, B., Helland, Å., Giannone, V., D'Amelio, A. M., Zhang, P., Mookerjee, B., & Johnson, B. E. (2017). Dabrafenib plus trametinib in patients with previously untreated BRAFV600E-mutant metastatic non-small-cell lung cancer: An open-label, phase 2 trial. *The Lancet. Oncology*, *18*(10), 1307–1316. https://doi.org/10.1016/S1470-2045(17)30679-4

Pokharel, Y. R., Saarela, J., Szwajda, A., Rupp, C., Rokka, A., Lal Kumar Karna, S., Teittinen, K., Corthals, G., Kallioniemi, O., Wennerberg, K., Aittokallio, T., & Westermarck, J. (2015). Relevance Rank Platform (RRP) for Functional Filtering of High Content Protein-Protein Interaction Data. *Molecular & Cellular Proteomics: MCP*, *14*(12), 3274–3283. https://doi.org/10.1074/mcp.M115.050773

Pusey, M., Bail, S., Xu, Y., Buiakova, O., Nestor, M., Yang, J.-J., & Rice, L. M. (2016). Inhibition of protein methylesterase 1 decreased cancerous phenotypes in endometrial adenocarcinoma cell lines and xenograft

tumor models. *Tumour Biology: The Journal of the International Society for Oncodevelopmental Biology and Medicine*, *37*(9), 11835–11842. https://doi.org/10.1007/s13277-016-5036-8

Puustinen, P., Junttila, M. R., Vanhatupa, S., Sablina, A. A., Hector, M. E., Teittinen, K., Raheem, O., Ketola, K., Lin, S., Kast, J., Haapasalo, H., Hahn, W. C., & Westermarck, J. (2009). PME-1 protects extracellular signal-regulated kinase pathway activity from protein phosphatase 2A-mediated inactivation in human malignant glioma. *Cancer Research*, *69*(7), 2870–2877. https://doi.org/10.1158/0008-5472.CAN-08-2760

Qin, W., Leonhardt, H., & Spada, F. (2011). Usp7 and Uhrf1 control ubiquitination and stability of the maintenance DNA methyltransferase Dnmt1. *Journal of Cellular Biochemistry*, *112*(2), 439–444. https://doi.org/10.1002/jcb.22998

Rajalingam, K., Schreck, R., Rapp, U. R., & Albert, Š. (2007). Ras oncogenes and their downstream targets. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1773*(8), 1177–1195. https://doi.org/10.1016/j.bbamcr.2007.01.012

Rangarajan, A., Hong, S. J., Gifford, A., & Weinberg, R. A. (2004). Species- and cell type-specific requirements for cellular transformation. *Cancer Cell*, *6*(2), 171–183. https://doi.org/10.1016/j.ccr.2004.07.009

Reisman, D. N., Sciarrotta, J., Wang, W., Funkhouser, W. K., & Weissman, B. E. (2003). Loss of BRG1/BRM in Human Lung Cancer Cell Lines and Primary Lung Cancers: Correlation with Poor Prognosis1. *Cancer Research*, *63*(3), 560–566.

Rhee, I., Bachman, K. E., Park, B. H., Jair, K.-W., Yen, R.-W. C., Schuebel, K. E., Cui, H., Feinberg, A. P., Lengauer, C., Kinzler, K. W., Baylin, S. B., & Vogelstein, B. (2002). DNMT1 and DNMT3b cooperate to silence genes in human cancer cells. *Nature*, *416*(6880), Article 6880. https://doi.org/10.1038/416552a

Rodriguez-Viciana, P., Warne, P. H., Dhand, R., Vanhaesebroeck, B., Gout, I., Fry, M. J., Waterfield, M. D., & Downward, J. (1994). Phosphatidylinositol-3-OH kinase direct target of Ras. *Nature*, *370*(6490), Article 6490. https://doi.org/10.1038/370527a0

Ross, J. F., Liu, X., & Dynlacht, B. D. (1999). Mechanism of transcriptional repression of E2F by the retinoblastoma tumor suppressor protein. *Molecular Cell*, *3*(2), 195–205. https://doi.org/10.1016/s1097-2765(00)80310-x

Rouleau, J., MacLeod, A. R., & Szyf, M. (1995). Regulation of the DNA Methyltransferase by the Ras-AP-1 Signaling Pathway (∗). *Journal of Biological Chemistry*, *270*(4), 1595–1601. https://doi.org/10.1074/jbc.270.4.1595

Rountree, M. R., Bachman, K. E., & Baylin, S. B. (2000). DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. *Nature Genetics*, *25*(3), 269–277. https://doi.org/10.1038/77023

Rudin, C. M., Brambilla, E., Faivre-Finn, C., & Sage, J. (2021). Small-cell lung cancer. *Nature Reviews Disease Primers*, *7*(1), Article 1. https://doi.org/10.1038/s41572-020-00235-0

Ruediger, R., Pham, H. T., & Walter, G. (2001). Disruption of protein phosphatase 2A subunit interaction in human cancers with mutations in the A alpha subunit gene. *Oncogene*, *20*(1), 10–15. https://doi.org/10.1038/sj.onc.1204059

Ruediger, R., Ruiz, J., & Walter, G. (2011). Human cancer-associated mutations in the Aα subunit of protein phosphatase 2A increase lung cancer incidence in Aα knock-in and knockout mice. *Molecular and Cellular Biology*, *31*(18), 3832–3844. https://doi.org/10.1128/MCB.05744-11

Ruvolo, P. P., Clark, W., Mumby, M., Gao, F., & May, W. S. (2002). A functional role for the B56 alpha-subunit of protein phosphatase 2A in ceramide-mediated regulation of Bcl2 phosphorylation status and function. *The Journal of Biological Chemistry*, *277*(25), 22847–22852. https://doi.org/10.1074/jbc.M201830200

Sablina, A. A., Hector, M., Colpaert, N., & Hahn, W. C. (2010). Identification of PP2A complexes and pathways involved in cell transformation. *Cancer Research*, *70*(24), 10474–10484. https://doi.org/10.1158/0008-5472.CAN-10-2855

Saddoughi, S. A., Gencer, S., Peterson, Y. K., Ward, K. E., Mukhopadhyay, A., Oaks, J., Bielawski, J., Szulc, Z. M., Thomas, R. J., Selvam, S. P., Senkal, C. E., Garrett-Mayer, E., De Palma, R. M., Fedarovich, D., Liu, A., Habib, A. A., Stahelin, R. V., Perrotti, D., & Ogretmen, B. (2013). Sphingosine analogue drug FTY720 targets I2PP2A/SET and mediates lung tumour suppression via activation of PP2A-RIPK1-dependent necroptosis. *EMBO Molecular Medicine*, *5*(1), 105–121. https://doi.org/10.1002/emmm.201201283

Sánchez-Molina, S., Estarás, C., Oliva, J. L., Akizu, N., Asensio-Juan, E., Rojas, J. M., & Martínez-Balbás, M. A. (2014). Regulation of CBP and Tip60 coordinates histone acetylation at local and global levels during Ras-induced transformation. *Carcinogenesis*, *35*(10), 2194–2202. https://doi.org/10.1093/carcin/bgu111

Sangodkar, J., Perl, A., Tohme, R., Kiselar, J., Kastrinsky, D. B., Zaware, N., Izadmehr, S., Mazhar, S., Wiredja, D. D., O'Connor, C. M., Hoon, D., Dhawan, N. S., Schlatzer, D., Yao, S., Leonard, D., Borczuk, A. C., Gokulrangan, G., Wang, L., Svenson, E., … Narla, G. (2017). Activation of tumor suppressor protein PP2A inhibits KRAS-driven tumor growth. *The Journal of Clinical Investigation*, *127*(6), 2081–2090. https://doi.org/10.1172/JCI89548

Santos, E., Martin-Zanca, D., Reddy, E. P., Pierotti, M. A., Della Porta, G., & Barbacid, M. (1984). Malignant Activation of a K-ras Oncogene in Lung Carcinoma But Not in Normal Tissue of the Same Patient. *Science*, *223*(4637), 661–664. https://doi.org/10.1126/science.6695174

Santos, E., Tronick, S. R., Aaronson, S. A., Pulciani, S., & Barbacid, M. (1982). T24 human bladder carcinoma oncogene is an activated form of the normal human homologue of BALB- and Harvey-MSV transforming genes. *Nature*, *298*(5872), 343–347. https://doi.org/10.1038/298343a0

Sanz-Álvarez, M., Cristóbal, I., Luque, M., Santos, A., Zazo, S., Madoz-Gúrpide, J., Caramés, C., Chiang, C.-M., García-Foncillas, J., Eroles, P., Albanell, J., & Rojo, F. (2021). Expression of Phosphorylated BRD4 Is Markedly Associated with the Activation Status of the PP2A Pathway and Shows a Strong Prognostic Value in Triple Negative Breast Cancer Patients. *Cancers*, *13*(6), 1246. https://doi.org/10.3390/cancers13061246

Sarkar, S., Abujamra, A. L., Loew, J. E., Forman, L. W., Perrine, S. P., & Faller, D. V. (2011). Histone deacetylase inhibitors reverse CpG methylation by regulating DNMT1 through ERK signaling. *Anticancer Research*, *31*(9), 2723–2732.

Sasaki, T., Koivunen, J., Ogino, A., Yanagita, M., Nikiforow, S., Zheng, W., Lathan, C., Marcoux, J. P., Du, J., Okuda, K., Capelletti, M., Shimamura, T., Ercan, D., Stumpfova, M., Xiao, Y., Weremowicz, S., Butaney, M., Heon, S., Wilner, K., … Jänne, P. A. (2011). A novel ALK secondary mutation and EGFR signaling cause resistance to ALK kinase inhibitors. *Cancer Research*, *71*(18), 6051–6060. https://doi.org/10.1158/0008-5472.CAN-11-1340

Scheffzek, K., Ahmadian, M. R., Kabsch, W., Wiesmüller, L., Lautwein, A., Schmitz, F., & Wittinghofer, A. (1997). The Ras-RasGAP complex: Structural basis for GTPase activation and its loss in oncogenic Ras mutants. *Science (New York, N.Y.)*, *277*(5324), 333–338. https://doi.org/10.1126/science.277.5324.333

Scolnick, E. M., Rands, E., Williams, D., & Parks, W. P. (1973). Studies on the Nucleic Acid Sequences of Kirsten Sarcoma Virus: A Model for Formation of a Mammalian RNA-Containing Sarcoma Virus. *Journal of Virology*, *12*(3), 458–463. https://doi.org/10.1128/jvi.12.3.458-463.1973

Scott, A., Song, J., Ewing, R., & Wang, Z. (2014). Regulation of protein stability of DNA methyltransferase 1 by post-translational modifications. *Acta Biochimica Et Biophysica Sinica*, *46*(3), 199–203. https://doi.org/10.1093/abbs/gmt146

Seligson, D. B., Horvath, S., McBrian, M. A., Mah, V., Yu, H., Tze, S., Wang, Q., Chia, D., Goodglick, L., & Kurdistani, S. K. (2009). Global levels of histone modifications predict prognosis in different cancers. *The American Journal of Pathology*, *174*(5), 1619–1628. https://doi.org/10.2353/ajpath.2009.080874

Semenova, E. A., Nagel, R., & Berns, A. (2015). Origins, genetic landscape, and emerging therapies of small cell lung cancer. *Genes & Development*, *29*(14), 1447–1462. https://doi.org/10.1101/gad.263145.115

Seo, S. B., McNamara, P., Heo, S., Turner, A., Lane, W. S., & Chakravarti, D. (2001a). Regulation of histone acetylation and transcription by INHAT, a human cellular complex containing the set oncoprotein. *Cell*, *104*(1), 119–130. https://doi.org/10.1016/s0092-8674(01)00196-9

Seo, S. B., McNamara, P., Heo, S., Turner, A., Lane, W. S., & Chakravarti, D. (2001b). Regulation of histone acetylation and transcription by INHAT, a human cellular complex containing the set oncoprotein. *Cell*, *104*(1), 119–130. https://doi.org/10.1016/s0092-8674(01)00196-9

Shao, D. D., Xue, W., Krall, E. B., Bhutkar, A., Piccioni, F., Wang, X., Schinzel, A. C., Sood, S., Rosenbluh, J., Kim, J. W., Zwang, Y., Roberts, T. M., Root, D. E., Jacks, T., & Hahn, W. C. (2014). KRAS and YAP1 converge to regulate EMT and tumor survival. *Cell*, *158*(1), 171–184. https://doi.org/10.1016/j.cell.2014.06.004

Shi, Y.-J., Matson, C., Lan, F., Iwase, S., Baba, T., & Shi, Y. (2005). Regulation of LSD1 histone demethylase activity by its associated factors. *Molecular Cell*, *19*(6), 857–864. https://doi.org/10.1016/j.molcel.2005.08.027

Shi, Y.-X., Sheng, D.-Q., Cheng, L., & Song, X.-Y. (2019). Current Landscape of Epigenetics in Lung Cancer: Focus on the Mechanism and Application. *Journal of Oncology*, *2019*, e8107318. https://doi.org/10.1155/2019/8107318

Shifteh, D., Sapir, T., Pahmer, M., Haimowitz, A., Goel, S., & Maitra, R. (2020). Protein Arginine Methyltransferase 5 as a Therapeutic Target for KRAS Mutated Colorectal Cancer. *Cancers*, *12*(8), 2091. https://doi.org/10.3390/cancers12082091

Shih, C., Shilo, B. Z., Goldfarb, M. P., Dannenberg, A., & Weinberg, R. A. (1979). Passage of phenotypes of chemically transformed cells via transfection of DNA and chromatin. *Proceedings of the National Academy of Sciences of the United States of America*, *76*(11), 5714–5718. https://doi.org/10.1073/pnas.76.11.5714

Shih, T. Y., Weeks, M. O., Young, H. A., & Scholnick, E. M. (1979). Identification of a sarcoma virus-coded phosphoprotein in nonproducer cells transformed by Kirsten or Harvey murine sarcoma virus. *Virology*, *96*(1), 64–79. https://doi.org/10.1016/0042-6822(79)90173-9

Shinjo, K., Okamoto, Y., An, B., Yokoyama, T., Takeuchi, I., Fujii, M., Osada, H., Usami, N., Hasegawa, Y., Ito, H., Hida, T., Fujimoto, N., Kishimoto, T., Sekido, Y., & Kondo, Y. (2012). Integrated analysis of genetic and epigenetic alterations reveals CpG island methylator phenotype associated with distinct clinical characters of lung adenocarcinoma. *Carcinogenesis*, *33*(7), 1277–1285. https://doi.org/10.1093/carcin/bgs154

Shu, S., Lin, C. Y., He, H. H., Witwicki, R. M., Tabassum, D. P., Roberts, J. M., Janiszewska, M., Huh, S. J., Liang, Y., Ryan, J., Doherty, E., Mohammed, H., Guo, H., Stover, D. G., Ekram, M. B., Brown, J., D'Santos, C., Krop, I. E., Dillon, D., … Polyak, K. (2016). Response and resistance to BET bromodomain inhibitors in triple-negative breast cancer. *Nature*, *529*(7586), 413–417. https://doi.org/10.1038/nature16508

Shvedunova, M., & Akhtar, A. (2022). Modulation of cellular processes by histone and non-histone protein acetylation. *Nature Reviews. Molecular Cell Biology*, *23*(5), 329–349. https://doi.org/10.1038/s41580-021-00441-y

Simpson, R. T. (1978). Structure of the chromatosome, a chromatin particle containing 160 base pairs of DNA and all the histones. *Biochemistry*, *17*(25), 5524–5531. https://doi.org/10.1021/bi00618a030

Smith, M. J., Neel, B. G., & Ikura, M. (2013). NMR-based functional profiling of RASopathies and oncogenic RAS mutations. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(12), 4574–4579. https://doi.org/10.1073/pnas.1218173110

Solomon, B., Bauer, T. M., Marinis, F. D., Felip, E., Goto, Y., Liu, G., Mazieres, J., Kim, D.-W., Mok, T., Polli, A., Thurm, H., Calella, A. M., Peltz, G., & Shaw, A. (2020). LBA2 Lorlatinib vs crizotinib in the first-line treatment of patients (pts) with advanced ALK-positive non-small cell lung cancer (NSCLC): Results of the phase III CROWN study. *Annals of Oncology*, *31*, S1180–S1181. https://doi.org/10.1016/j.annonc.2020.08.2282

Solomon, B. J., Kim, D.-W., Wu, Y.-L., Nakagawa, K., Mekhail, T., Felip, E., Cappuzzo, F., Paolini, J., Usari, T., Tang, Y., Wilner, K. D., Blackhall, F., & Mok, T. S. (2018). Final Overall Survival Analysis

From a Study Comparing First-Line Crizotinib Versus Chemotherapy in ALK-Mutation-Positive Non-Small-Cell Lung Cancer. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, *36*(22), 2251–2258. https://doi.org/10.1200/JCO.2017.77.4794

Song, J. S., Kim, Y. S., Kim, D. K., Park, S. il, & Jang, S. J. (2012). Global histone modification pattern associated with recurrence and disease-free survival in non-small cell lung cancer patients. *Pathology International*, *62*(3), 182–190. https://doi.org/10.1111/j.1440-1827.2011.02776.x

Song, Y., Dagil, L., Fairall, L., Robertson, N., Wu, M., Ragan, T. J., Savva, C. G., Saleh, A., Morone, N., Kunze, M. B. A., Jamieson, A. G., Cole, P. A., Hansen, D. F., & Schwabe, J. W. R. (2020). Mechanism of Crosstalk between the LSD1 Demethylase and HDAC1 Deacetylase in the CoREST Complex. *Cell Reports*, *30*(8), 2699-2711.e8. https://doi.org/10.1016/j.celrep.2020.01.091

Standfield, L., Weston, A. R., Barraclough, H., Van Kooten, M., & Pavlakis, N. (2011). Histology as a treatment effect modifier in advanced non-small cell lung cancer: A systematic review of the evidence. *Respirology (Carlton, Vic.)*, *16*(8), 1210–1220. https://doi.org/10.1111/j.1440-1843.2011.02025.x

Strack, S., Zaucha, J. A., Ebner, F. F., Colbran, R. J., & Wadzinski, B. E. (1998). Brain protein phosphatase 2A: Developmental regulation and distinct cellular and subcellular localization by B subunits. *The Journal of Comparative Neurology*, *392*(4), 515–527.

Strader, C. R., Pearce, C. J., & Oberlies, N. H. (2011). Fingolimod (FTY720): A recently approved multiple sclerosis drug based on a fungal secondary metabolite. *Journal of Natural Products*, *74*(4), 900–907. https://doi.org/10.1021/np2000528

Strahl, B. D., Briggs, S. D., Brame, C. J., Caldwell, J. A., Koh, S. S., Ma, H., Cook, R. G., Shabanowitz, J., Hunt, D. F., Stallcup, M. R., & Allis, C. D. (2001). Methylation of histone H4 at arginine 3 occurs in vivo and is mediated by the nuclear receptor coactivator PRMT1. *Current Biology: CB*, *11*(12), 996–1000. https://doi.org/10.1016/s0960-9822(01)00294-9

Sucharov, C. C., Langer, S., Bristow, M., & Leinwand, L. (2006). Shuttling of HDAC5 in H9C2 cells regulates YY1 function through CaMKIV/PKD and PP2A. *American Journal of Physiology. Cell Physiology*, *291*(5), C1029-1037. https://doi.org/10.1152/ajpcell.00059.2006

Sulewska, A., Pilz, L., Manegold, C., Ramlau, R., Charkiewicz, R., & Niklinski, J. (2023). A Systematic Review of Progress toward Unlocking the Power of Epigenetics in NSCLC: Latest Updates and Perspectives. *Cells*, *12*(6), 905. https://doi.org/10.3390/cells12060905

Sunahori, K., Nagpal, K., Hedrich, C. M., Mizui, M., Fitzgerald, L. M., & Tsokos, G. C. (2013). The catalytic subunit of protein phosphatase 2A (PP2Ac) promotes DNA hypomethylation by suppressing the phosphorylated mitogen-activated protein kinase/extracellular signal-regulated kinase (ERK) kinase (MEK)/phosphorylated ERK/DNMT1 protein pathway in T-cells from controls and systemic lupus erythematosus patients. *The Journal of Biological Chemistry*, *288*(30), 21936–21944. https://doi.org/10.1074/jbc.M113.467266

Suresh, S., Huard, S., Brisson, A., Némati, F., Dakroub, R., Poulard, C., Ye, M., Martel, E., Reyes, C., Silvestre, D. C., Meseure, D., Nicolas, A., Gentien, D., Fayyad-Kazan, H., Le Romancer, M., Decaudin, D., Roman-Roman, S., & Dubois, T. (2022). PRMT1 Regulates EGFR and Wnt Signaling Pathways and Is a Promising Target for Combinatorial Treatment of Breast Cancer. *Cancers*, *14*(2), 306. https://doi.org/10.3390/cancers14020306

Šutić, M., Vukić, A., Baranašić, J., Försti, A., Džubur, F., Samaržija, M., Jakopović, M., Brčić, L., & Knežević, J. (2021). Diagnostic, Predictive, and Prognostic Biomarkers in Non-Small Cell Lung Cancer (NSCLC) Management. *Journal of Personalized Medicine*, *11*(11), 1102. https://doi.org/10.3390/jpm11111102

Suzuki, M., Sunaga, N., Shames, D. S., Toyooka, S., Gazdar, A. F., & Minna, J. D. (2004). RNA interference-mediated knockdown of DNA methyltransferase 1 leads to promoter demethylation and gene re-expression in human lung and breast cancer cells. *Cancer Research*, *64*(9), 3137–3143. https://doi.org/10.1158/0008-5472.can-03-3046

Szyf, M., Theberge, J., & Bozovic, V. (1995). Ras Induces a General DNA Demethylation Activity in Mouse Embryonal P19 Cells (∗). *Journal of Biological Chemistry*, *270*(21), 12690–12696. https://doi.org/10.1074/jbc.270.21.12690

Tagal, V., Wei, S., Zhang, W., Brekken, R. A., Posner, B. A., Peyton, M., Girard, L., Hwang, T., Wheeler, D. A., Minna, J. D., White, M. A., Gazdar, A. F., & Roth, M. G. (2017). SMARCA4-inactivating mutations increase sensitivity to Aurora kinase A inhibitor VX-680 in non-small cell lung cancers. *Nature Communications*, *8*, 14098. https://doi.org/10.1038/ncomms14098

Tahiliani, M., Koh, K. P., Shen, Y., Pastor, W. A., Bandukwala, H., Brudno, Y., Agarwal, S., Iyer, L. M., Liu, D. R., Aravind, L., & Rao, A. (2009). Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science (New York, N.Y.)*, *324*(5929), 930–935. https://doi.org/10.1126/science.1170116

Tan, J., Lee, P. L., Li, Z., Jiang, X., Lim, Y. C., Hooi, S. C., & Yu, Q. (2010). B55β-associated PP2A complex controls PDK1-directed myc signaling and modulates rapamycin sensitivity in colorectal cancer. *Cancer Cell*, *18*(5), 459–471. https://doi.org/10.1016/j.ccr.2010.10.021

Tanimoto, A., Takeuchi, S., Arai, S., Fukuda, K., Yamada, T., Roca, X., Ong, S. T., & Yano, S. (2017). Histone Deacetylase 3 Inhibition Overcomes BIM Deletion Polymorphism–Mediated Osimertinib Resistance in EGFR-Mutant Lung Cancer. *Clinical Cancer Research*, *23*(12), 3139–3149. https://doi.org/10.1158/1078-0432.CCR-16-2271

Teneng, I., Tellez, C. S., Picchi, M. A., Klinge, D. M., Yingling, C. M., Snider, A. M., Liu, Y., & Belinsky, S. A. (2015). Global identification of genes targeted by DNMT3b for epigenetic silencing in lung cancer. *Oncogene*, *34*(5), Article 5. https://doi.org/10.1038/onc.2013.580

Thai, A. A., Solomon, B. J., Sequist, L. V., Gainor, J. F., & Heist, R. S. (2021). Lung cancer. *The Lancet*, *398*(10299), 535–554. https://doi.org/10.1016/S0140-6736(21)00312-3

Toska, E., Osmanbeyoglu, H. U., Castel, P., Chan, C., Hendrickson, R. C., Elkabets, M., Dickler, M. N., Scaltriti, M., Leslie, C. S., Armstrong, S. A., & Baselga, J. (2017). PI3K pathway regulates ER-dependent transcription in breast cancer through the epigenetic regulator KMT2D. *Science (New York, N.Y.)*, *355*(6331), 1324–1330. https://doi.org/10.1126/science.aah6893

Treviño, L. S., Wang, Q., & Walker, C. L. (2015). Phosphorylation of Epigenetic "Readers, Writers and Erasers": Implications for Developmental Reprogramming and the Epigenetic Basis for Health and Disease. *Progress in Biophysics and Molecular Biology*, *118*(0), 8–13. https://doi.org/10.1016/j.pbiomolbio.2015.02.013

Trouche, D., Le Chalony, C., Muchardt, C., Yaniv, M., & Kouzarides, T. (1997). RB and hbrm cooperate to repress the activation functions of E2F1. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(21), 11268–11273. https://doi.org/10.1073/pnas.94.21.11268

Tsai, S.-C., & Seto, E. (2002). Regulation of histone deacetylase 2 by protein kinase CK2. *The Journal of Biological Chemistry*, *277*(35), 31826–31833. https://doi.org/10.1074/jbc.M204149200

Tyagi, M., Imam, N., Verma, K., & Patel, A. K. (2016). Chromatin remodelers: We are the drivers!! *Nucleus (Austin, Tex.)*, *7*(4), 388–404. https://doi.org/10.1080/19491034.2016.1211217

Ugi, S., Imamura, T., Ricketts, W., & Olefsky, J. M. (2002). Protein phosphatase 2A forms a molecular complex with Shc and regulates Shc tyrosine phosphorylation and downstream mitogenic signaling. *Molecular and Cellular Biology*, *22*(7), 2375–2387. https://doi.org/10.1128/MCB.22.7.2375-2387.2002

Van Den Broeck, A., Brambilla, E., Moro-Sibilot, D., Lantuejoul, S., Brambilla, C., Eymin, B., Khochbin, S., & Gazzeri, S. (2008). Loss of Histone H4K20 Trimethylation Occurs in Preneoplasia and Influences Prognosis of Non–Small Cell Lung Cancer. *Clinical Cancer Research*, *14*(22), 7237–7245. https://doi.org/10.1158/1078-0432.CCR-08-0869

Vetter, I. R., & Wittinghofer, A. (2001). The guanine nucleotide-binding switch in three dimensions. *Science (New York, N.Y.)*, *294*(5545), 1299–1304. https://doi.org/10.1126/science.1062023

Walter, D. M., Venancio, O. S., Buza, E. L., Tobias, J. W., Deshpande, C., Gudiel, A. A., Kim-Kiselak, C., Cicchini, M., Yates, T. J., & Feldser, D. M. (2017). Systematic In Vivo Inactivation of Chromatin-Regulating Enzymes Identifies Setd2 as a Potent Tumor Suppressor in Lung Adenocarcinoma. *Cancer Research*, *77*(7), 1719–1729. https://doi.org/10.1158/0008-5472.CAN-16-2159

Wandzioch, E., Pusey, M., Werda, A., Bail, S., Bhaskar, A., Nestor, M., Yang, J.-J., & Rice, L. M. (2014). PME-1 modulates protein phosphatase 2A activity to promote the malignant phenotype of endometrial cancer cells. *Cancer Research*, *74*(16), 4295–4305. https://doi.org/10.1158/0008-5472.CAN-13-3130

Wang, A. H., & Yang, X. J. (2001). Histone deacetylase 4 possesses intrinsic nuclear import and export signals. *Molecular and Cellular Biology*, *21*(17), 5992–6005. https://doi.org/10.1128/MCB.21.17.5992-6005.2001

Wang, J. D., Takahara, S., Nonomura, N., Ichimaru, N., Toki, K., Azuma, H., Matsumiya, K., Okuyama, A., & Suzuki, S. (1999). Early induction of apoptosis in androgen-independent prostate cancer cell line by FTY720 requires caspase-3 activation. *The Prostate*, *40*(1), 50–55. https://doi.org/10.1002/(sici)1097-0045(19990615)40:1<50::aid-pros6>3.0.co;2-n

Wang, J., Hevi, S., Kurash, J. K., Lei, H., Gay, F., Bajko, J., Su, H., Sun, W., Chang, H., Xu, G., Gaudet, F., Li, E., & Chen, T. (2009). The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation. *Nature Genetics*, *41*(1), 125–129. https://doi.org/10.1038/ng.268

Wang, R., Li, Q., Helfer, C. M., Jiao, J., & You, J. (2012). Bromodomain protein Brd4 associated with acetylated chromatin is important for maintenance of higher-order chromatin structure. *The Journal of Biological Chemistry*, *287*(14), 10738–10752. https://doi.org/10.1074/jbc.M111.323493

Wang, S. S., Esplin, E. D., Li, J. L., Huang, L., Gazdar, A., Minna, J., & Evans, G. A. (1998). Alterations of the PPP2R1B gene in human lung and colon cancer. *Science (New York, N.Y.)*, *282*(5387), 284–287. https://doi.org/10.1126/science.282.5387.284

Wang, X., Bajaj, R., Bollen, M., Peti, W., & Page, R. (2016). Expanding the PP2A Interactome by Defining a B56-Specific SLiM. *Structure (London, England: 1993)*, *24*(12), 2174–2181. https://doi.org/10.1016/j.str.2016.09.010

Wang, X., Garvanska, D. H., Nasa, I., Ueki, Y., Zhang, G., Kettenbach, A. N., Peti, W., Nilsson, J., & Page, R. (2020). A dynamic charge-charge interaction modulates PP2A:B56 substrate recruitment. *eLife*, *9*, e55966. https://doi.org/10.7554/eLife.55966

Wang, Y., Yang, R., Gu, J., Yin, X., Jin, N., Xie, S., Wang, Y., Chang, H., Qian, W., Shi, J., Iqbal, K., Gong, C.-X., Cheng, C., & Liu, F. (2015). Cross talk between PI3K-AKT-GSK-3β and PP2A pathways determines tau hyperphosphorylation. *Neurobiology of Aging*, *36*(1), 188–200. https://doi.org/10.1016/j.neurobiolaging.2014.07.035

Wang, Z., Tang, F., Hu, P., Wang, Y., Gong, J., Sun, S., & Xie, C. (2016). HDAC6 promotes cell proliferation and confers resistance to gefitinib in lung adenocarcinoma. *Oncology Reports*, *36*(1), 589–597. https://doi.org/10.3892/or.2016.4811

Watson, A. A., Mahajan, P., Mertens, H. D. T., Deery, M. J., Zhang, W., Pham, P., Du, X., Bartke, T., Zhang, W., Edlich, C., Berridge, G., Chen, Y., Burgess-Brown, N. A., Kouzarides, T., Wiechens, N., Owen-Hughes, T., Svergun, D. I., Gileadi, O., & Laue, E. D. (2012). The PHD and chromo domains regulate the ATPase activity of the human chromatin remodeler CHD4. *Journal of Molecular Biology*, *422*(1), 3–17. https://doi.org/10.1016/j.jmb.2012.04.031

Weeks, K. L., Ranieri, A., Karaś, A., Bernardo, B. C., Ashcroft, A. S., Molenaar, C., McMullen, J. R., & Avkiran, M. (2017). β-Adrenergic Stimulation Induces Histone Deacetylase 5 (HDAC5) Nuclear Accumulation in Cardiomyocytes by B55α-PP2A-Mediated Dephosphorylation. *Journal of the American Heart Association*, *6*(4), e004861. https://doi.org/10.1161/JAHA.116.004861

Wegel, E., & Shaw, P. (2005). Gene activation and deactivation related changes in the three-dimensional structure of chromatin. *Chromosoma*, *114*(5), 331–337. https://doi.org/10.1007/s00412-005-0015-7

Wei, Y., Maximov, V., Morrissy, S. A., Taylor, M. D., Pallas, D. C., & Kenney, A. M. (2019). P53 Function Is Compromised by Inhibitor 2 of Phosphatase 2A in Sonic Hedgehog Medulloblastoma. *Molecular Cancer Research: MCR*, *17*(1), 186–198. https://doi.org/10.1158/1541-7786.MCR-18-0485

Westermarck, J. (2018). Targeted therapies don't work for a reason; the neglected tumor suppressor phosphatase PP2A strikes back. *The FEBS Journal*, *285*(22), 4139–4145. https://doi.org/10.1111/febs.14617

Westermarck, J., & Neel, B. G. (2020). Piecing Together a Broken Tumor Suppressor Phosphatase for Cancer Therapy. *Cell*, *181*(3), 514–517. https://doi.org/10.1016/j.cell.2020.04.005

Witte, T., Plass, C., & Gerhauser, C. (2014). Pan-cancer patterns of DNA methylation. *Genome Medicine*, *6*(8), 66. https://doi.org/10.1186/s13073-014-0066-6

Wlodarchak, N., & Xing, Y. (2016). PP2A as a master regulator of the cell cycle. *Critical Reviews in Biochemistry and Molecular Biology*, *51*(3), 162–184. https://doi.org/10.3109/10409238.2016.1143913

Wu, S.-Y., Lee, A.-Y., Lai, H.-T., Zhang, H., & Chiang, C.-M. (2013). Phospho switch triggers Brd4 chromatin binding and activator recruitment for gene-specific targeting. *Molecular Cell*, *49*(5), 843–857. https://doi.org/10.1016/j.molcel.2012.12.006

Wu, X.-Y., Chen, H.-C., Li, W.-W., Yan, J.-D., & Lv, R.-Y. (2020). DNMT1 promotes cell proliferation via methylating hMLH1 and hMSH2 promoters in EGFR-mutated non-small cell lung cancer. *Journal of Biochemistry*, *168*(2), 151–157. https://doi.org/10.1093/jb/mvaa034

Wutz, A., Smrzka, O. W., Schweifer, N., Schellander, K., Wagner, E. F., & Barlow, D. P. (1997). Imprinted expression of the Igf2r gene depends on an intronic CpG island. *Nature*, *389*(6652), 745–749. https://doi.org/10.1038/39631

Xiao, P., Chen, J., Zhou, F., Lu, C., Yang, Q., Tao, G., Tao, Y., & Chen, J. (2014). Methylation of P16 in exhaled breath condensate for diagnosis of non-small cell lung cancer. *Lung Cancer*, *83*(1), 56–60. https://doi.org/10.1016/j.lungcan.2013.09.008

Xing, Y., Li, Z., Chen, Y., Stock, J. B., Jeffrey, P. D., & Shi, Y. (2008). Structural mechanism of demethylation and inactivation of protein phosphatase 2A. *Cell*, *133*(1), 154–163. https://doi.org/10.1016/j.cell.2008.02.041

Xing, Y., Xu, Y., Chen, Y., Jeffrey, P. D., Chao, Y., Lin, Z., Li, Z., Strack, S., Stock, J. B., & Shi, Y. (2006). Structure of protein phosphatase 2A core enzyme bound to tumor-inducing toxins. *Cell*, *127*(2), 341–353. https://doi.org/10.1016/j.cell.2006.09.025

Xu, Y., Chen, Y., Zhang, P., Jeffrey, P. D., & Shi, Y. (2008). Structure of a protein phosphatase 2A holoenzyme: Insights into B55-mediated Tau dephosphorylation. *Molecular Cell*, *31*(6), 873–885. https://doi.org/10.1016/j.molcel.2008.08.006

Xu, Y., Xing, Y., Chen, Y., Chao, Y., Lin, Z., Fan, E., Yu, J. W., Strack, S., Jeffrey, P. D., & Shi, Y. (2006). Structure of the protein phosphatase 2A holoenzyme. *Cell*, *127*(6), 1239–1251. https://doi.org/10.1016/j.cell.2006.11.033

Xue, Y., Meehan, B., Fu, Z., Wang, X. Q. D., Fiset, P. O., Rieker, R., Levins, C., Kong, T., Zhu, X., Morin, G., Skerritt, L., Herpel, E., Venneti, S., Martinez, D., Judkins, A. R., Jung, S., Camilleri-Broet, S., Gonzalez, A. V., Guiot, M.-C., … Huang, S. (2019). SMARCA4 loss is synthetic lethal with CDK4/6 inhibition in non-small cell lung cancer. *Nature Communications*, *10*(1), 557. https://doi.org/10.1038/s41467-019-08380-1

Y, S., F, L., C, M., P, M., Jr, W., Pa, C., Ra, C., & Y, S. (2004). Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell*, *119*(7). https://doi.org/10.1016/j.cell.2004.12.012

Yamada, T., Amann, J. M., Tanimoto, A., Taniguchi, H., Shukuya, T., Timmers, C., Yano, S., Shilo, K., & Carbone, D. P. (2018). Histone Deacetylase Inhibition Enhances the Antitumor Activity of a MEK Inhibitor in Lung Cancer Cells Harboring RAS Mutations. *Molecular Cancer Therapeutics*, *17*(1), 17–25. https://doi.org/10.1158/1535-7163.MCT-17-0146

Yamashita, D., Moriuchi, T., Osumi, T., & Hirose, F. (2016). Transcription Factor hDREF Is a Novel SUMO E3 Ligase of Mi2α. *The Journal of Biological Chemistry*, *291*(22), 11619–11634. https://doi.org/10.1074/jbc.M115.713370

Yamazoe, S., Tom, J., Fu, Y., Wu, W., Zeng, L., Sun, C., Liu, Q., Lin, J., Lin, K., Fairbrother, W. J., & Staben, S. T. (2020). Heterobifunctional Molecules Induce Dephosphorylation of Kinases-A Proof of Concept Study. *Journal of Medicinal Chemistry*, *63*(6), 2807–2813. https://doi.org/10.1021/acs.jmedchem.9b01167

Yang, S. I., Lickteig, R. L., Estes, R., Rundell, K., Walter, G., & Mumby, M. C. (1991). Control of protein phosphatase 2A by simian virus 40 small-t antigen. *Molecular and Cellular Biology*, *11*(4), 1988–1995. https://doi.org/10.1128/mcb.11.4.1988-1995.1991

Yang, T., Jian, W., Luo, Y., Fu, X., Noguchi, C., Bungert, J., Huang, S., & Qiu, Y. (2012). Acetylation of histone deacetylase 1 regulates NuRD corepressor complex activity. *The Journal of Biological Chemistry*, *287*(48), 40279–40291. https://doi.org/10.1074/jbc.M112.349704