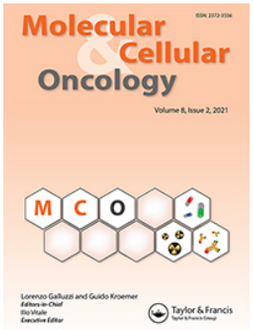

Molecular & Cellular Oncology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/kmco20

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To cite this article: Eeva-Liisa Eskelinen, Shun Kageyama & Masaaki Komatsu (2021) p62/SQSTM1 droplets initiate autophagosome biogenesis and oxidative stress control, Molecular & Cellular Oncology, 8:2, 1890990, DOI: 10.1080/23723556.2021.1890990

To link to this article: https://doi.org/10.1080/23723556.2021.1890990

Published online: 09 Mar 2021.

MOLECULAR & CELLULAR ONCOLOGY
2021, VOL. 8, NO. 2, e1890990 (3 pages)
https://doi.org/10.1080/23723556.2021.1890990

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AUTHOR'S VIEWS

p62/SQSTM1 droplets initiate autophagosome biogenesis and oxidative stress control

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ABSTRACT

Selective autophagy contributes to the degradation of condensates, such as sequestosome 1-bodies, also called p62/SQSTM1-bodies. We showed that endogenous p62 forms gel-like structures, which serve as platforms for autophagosome formation and nuclear factor erythroid 2-related factor 2 (NRF2) activation. Further, p62-mediated NRF2 activation is not cytotoxic, but combination of NRF2 activation with impaired bulk and selective autophagy causes liver injury.

ARTICLE HISTORY
Received 5 February 2021
Revised 10 February 2021
Accepted 11 February 2021

KEYWORDS
p62/SQSTM1; liquid-liquid phase separation; autophagy; oxidative stress; LC3; GABARAP; NRF2; KEAP1

Autophagy is a fundamental biological process that contributes to cytoplasmic quality control and cellular metabolism. Autophagy and oxidative stress play a crucial role in multiple diseases including cancer, inflammatory disorders, and neurodegenerative diseases.[1,2] Autophagy can be both nonselective and selective with respect to the cargo. While a lot is known about the molecular mechanisms of selective autophagy, fewer studies have specifically addressed its membrane dynamics and physiological roles. We addressed these issues for the selective autophagy of the autophagy adaptor protein p62, also known as sequestosome 1 (SQSTM1).[3] p62-bodies have been identified in liver disorders including alcoholic hepatitis, nonalcoholic steatohepatitis, and hepatocellular carcinoma, and they are considered cellular aggregates or inclusion bodies. However, p62-bodies were recently demonstrated to be reversible structures that maintain internal fluidity and are functionally active in biochemical reactions.[4,5] Thus, they represent a type of liquid droplets. Droplet formation through liquid–liquid phase separation is a stress response against external disturbances such as harsh environmental conditions. However, the physiological roles of p62-droplets remained unknown. Our work showed that p62-bodies are functional gels that regulate autophagosome formation and oxidative stress response, and that impaired clearance of p62-gels abnormally enhances the response to oxidative stress. Our report facilitates the discovery of new therapeutic targets to modulate the autophagy pathway in disease and to combat oxidative stress.

Our results showed that p62-bodies are low-liquidity gels where p62 is phosphorylated at Ser349 and Ser403. The gels also contained ubiquitin, neighbor of BRCA1 gene 1 protein (NBR1), and core autophagy-related proteins including FAK family kinase-interacting protein of 200 kDa (FIP200), Unc-51 like autophagy activating kinase 1 (ULK1), WD repeat domain phosphoinositide-interacting protein 2 (WIPI2), and autophagy-related 16L1 (ATG16L1). We showed that multiple autophagosomes form on and around the p62-gels. Unlike in nonselective autophagy and mitophagy, the morphology of the nascent autophagosome membranes was very complex, suggesting membrane elongation was accelerated by the p62-gels. Many of the p62 gels had multiple double membranes around them. This may be due to the high amount of available binding sites for the core autophagy protein FIP200 on the p62-gel. FIP200 has been shown to bind to phosphorylated p62 on the condensate in order to promote autophagosome formation.[6]

ATG8-protein family consists of the microtubule-associated protein 1 light chain 3 (LC3) and γ-amino butyric acid receptor-associated protein (GABARAP) families. ATG8-proteins localize to the membranes of forming autophagosomes and are widely used as autophagy markers. Importantly, our data indicated that the interaction of ATG8-proteins on the forming autophagosome membranes with p62 in the gel determined the orientation of the nascent autophagosomes. To demonstrate this, we used a probe, called HyD-LIR,[7] consisting of a short hydrophobic domain of Aplysia phosphodiesterase 4 short-form (HyD); LC3-interacting region (LIR) of tumor protein p53-inducible nuclear protein 2 (TP53INP2); and a fluorescent protein. The HyD-LIR probe efficiently binds to the lipidated forms of ATG8-proteins, LC3-II, and GABARAP-II, localizing on the inner membrane of autophagosomes, thus competing with binding of the p62-gel with LC3/GABARAP. However, the probe does not affect autophagosome formation. Our results showed that if p62 was able to interact with LC3/GABARAP, p62-gel was engulfed by the forming autophagosome, while when binding was not possible, the autophagosome formed next to the p62-gel but did not engulf it (Figure 1). Thus, the interaction of p62 with LC3 and/or GABARAP is

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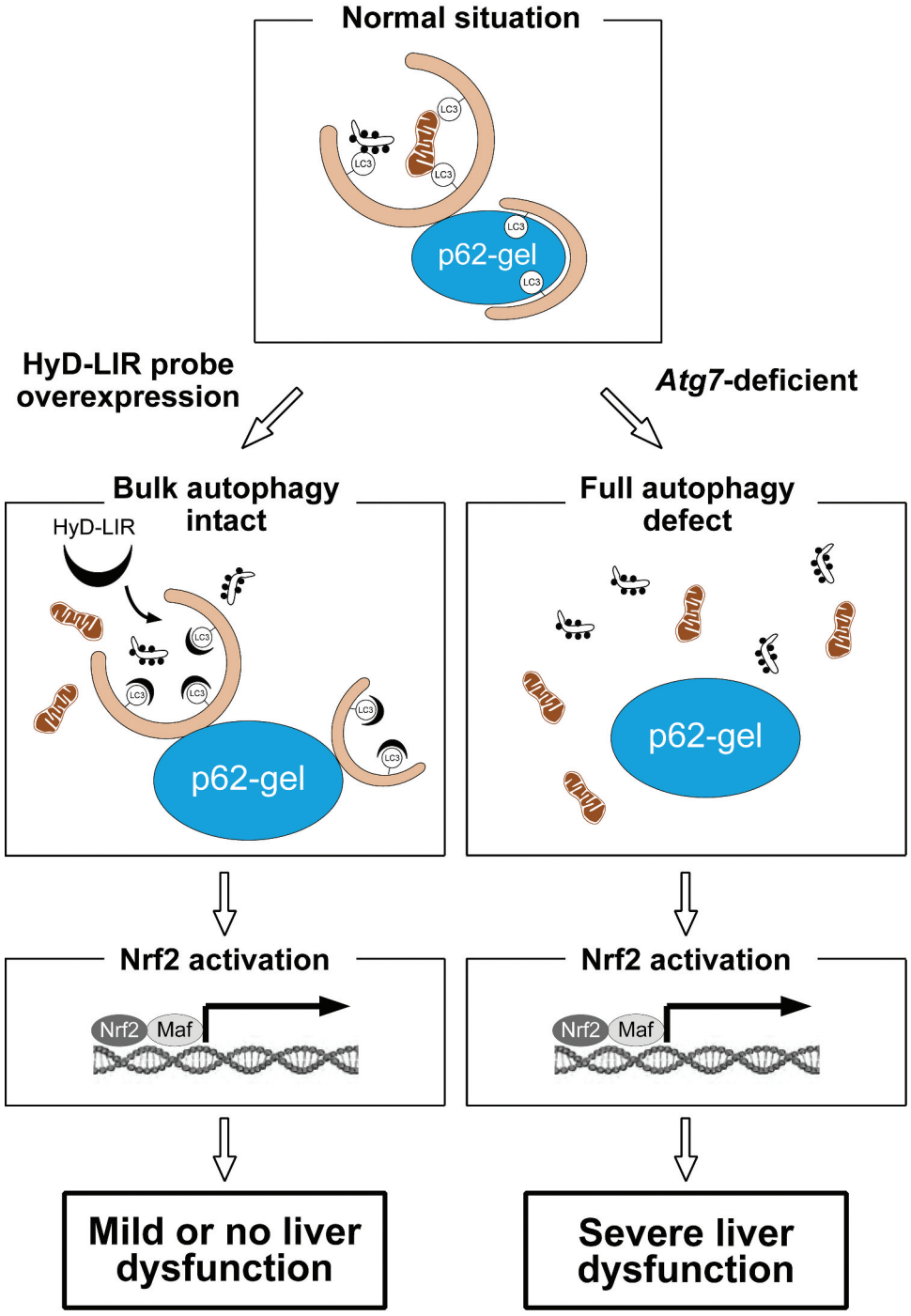

Figure 1. Autophagosome formation and antioxidative signaling initiation at p62 gels. In the normal situation, mitochondria (brown symbols) and endoplasmic reticulum (black-and-white symbol) can be sequestered randomly by basal autophagy as well as by selective autophagy via autophagy adaptors. In the presence of HyD-LIR, bulk autophagy is intact but selective autophagy is inhibited, while in Atg7-deficient conditions, both basal and selective autophagy are defective. Nrf2 is activated in both conditions, while only Atg7-deficiency causes liver injury.

required for selective autophagy of p62-gels. However, p62-gels accelerate autophagosome formation even if the autophagosomes would not sequester the p62-gel. Our results are fully in agreement with another recent report showing that p62–LC3 interaction on the p62-droplets controls the direction of autophagosome formation.[8]

Our results further demonstrated that p62-gels play a central role in oxidative stress response. Nuclear factor erythroid 2-related factor 2 (NRF2) is a basic leucine zipper (bZIP) transcription factor, and its heterodimer with small musculoaponeurotic fibrosarcoma (MAF) proteins controls the expression of proteins that protect against oxidative damage triggered by injury and inflammation. The tumor suppressor Kelch-like ECH-associated protein 1 (KEAP1), which interacts with Ser349-phosphorylated p62, is an adaptor of cullin3-based ubiquitin ligase for NRF2. We developed a small compound that inhibits p62-KEAP1 binding, and showed that KEAP1 reversibly translocated to the p62-gels in a p62-binding dependent manner. A p62 mutant defective in KEAP1 binding exhibited gel formation similar to wild-

type p62 but was not able to activate NRF2. Thus, p62-gels act in oxidative stress response through the reversible sequestration of KEAP1 (Figure 1).

Importantly, our results also revealed an in vivo role for the ATG8-interaction-dependent selective autophagy. We generated mice with liver-specific overexpression of the HyD-LIR probe, which inhibits selective autophagic sequestration of p62-gels. These mice showed an impaired turnover of p62-gels, which lead to Nrf2 hyperactivation and overexpression of Nrf2 target genes including antioxidant genes in the liver. However, the Nrf2 activation did not exert any cytotoxic effects on the liver. Instead, combination of Nrf2 activation with fully impaired bulk and selective autophagy (such as in Atg7-deficient liver) caused liver injury. In conclusion, severe liver injury occurs when both anabolism (Nrf2 activation) and catabolism (autophagy) are concomitantly deregulated.

Funding

S. K. is supported by a Grant-in-Aid for Scientific Research (C) (20K06549). M.K. is supported by a Grant-in-Aid for Scientific Research on Innovative Areas (19H05706), a Grant-in-Aid for Scientific Research (B) (18H02611), the Japan Society for the Promotion of Science (an A3 foresight program), Japan Agency for Medical Research and Development (AMED-CREST) and the Takeda Science Foundation. The work was supported by JSPS KAKENHI Grant Number JP 16H06276 (AdAMS). E.L.E. was supported by the Academy of Finland (grant No 286787) and Magnus Ehrnrooth Foundation.

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