**Mammalian autophagy degrades nuclear constituents in response to tumorigenic stress**

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**During autophagy, double-membrane autophagosomes are observed in the cytoplasm. Thus, extensive studies have focused on autophagic turnover of cytoplasmic material. Whether autophagy has a role in degrading nuclear constituents is poorly understood. We reveal that the autophagy protein LC3/Atg8 directly interacts with the nuclear lamina protein LMNB1 (lamin B1), and binds to LMN/lamin-associated chromatin domains (LADs). Through these interactions, autophagy specifically mediates destruction of nuclear lamina during tumorigenic stress, such as by activated oncogenes and DNA damage. This nuclear lamina degradation upon aberrant cellular stress impairs cell proliferation by inducing cellular senescence, a stable form of cell-cycle arrest and a tumor-suppressive mechanism. Our findings thus suggest that, in response to cancer-promoting stress, autophagy degrades nuclear material to drive cellular senescence, as a means to restrain tumorigenesis. Our work provokes a new direction in studying the role of autophagy in the nucleus and in tumor suppression.**

Understanding the role of autophagy in nuclear degradation has been elusive. In yeast, piecemeal microautophagy of the nucleus and Atg39-mediated nucleophagy have been reported. In certain binuclear or multinuclear eukaryotes, macroautophagy can degrade one of the nuclei. In mammals, macroautophagy (hereafter autophagy) mediates digestion of genetically mutated LMNs. Additionally, autophagy recognizes and degrades micronuclei in cancer cells. However, in broader physiology, whether autophagy in normal cells and tissues can degrade nuclear material is not understood. Along these lines, it is intriguing that several autophagy proteins are present in the nucleus, including LC3, ATG5, and ATG7.

We became interested in this topic during our study of cellular senescence. Senescence is an important cell state relating to both human aging and cancer. Senescence contributes to age-related diseases, as senescent cells are found in increased numbers in aged tissues, which are thought to impair tissue renewal and cause chronic inflammation. In contrast, senescence restrains tumorigenesis, by inducing cell-cycle arrest and directing the immune-mediated clearance of premalignant cells.

Senescence is associated with profound alterations in nuclear architecture and the epigenome. Strikingly, several nuclear proteins are lost during senescence, including nuclear lamina components. The nuclear lamina is a cytoskeleton meshwork beneath the nuclear envelope, composed of nuclear lamins—including LMNA (lamin A/C), LMNB1, LMNB2, and their associated proteins. This lamina structure provides the nucleus with mechanical strength as well as regulating the higher order organization of chromatin. The regions where chromatin contacts with the lamina are called LADs and are predominantly comprised of transcriptionally inactive heterochromatin. Upon induction of senescence, the downregulation of nuclear lamina proteins can occur within days. However, nuclear LMNs are among the most abundant and long-lived proteins in mammalian cells: their half-life can be as long as several months, according to quantitative mass spectrometry analyses. Thus, we reasoned that a degradation mechanism must exist to degrade these nuclear structural proteins during senescence.

Our earlier work, by Ivanov et al, discovered that the loss of nuclear material during senescence is through a nucleus-to-cytoplasm transport, forming cytoplasmic chromatin fragments that are targeted by the lysosomes. These observations established a foundation for our current work to directly investigate the role of autophagy. If the nuclear proteins are targeted by autophagy, one would expect an interaction of these components with autophagy proteins. To test this, we performed co-immunoprecipitation with a number of autophagy proteins, which led to the discovery of an LC3-LMNB1 interaction. Several groups have performed autophagy protein interactome studies; however, virtually no nuclear proteins were identified. This is likely because typical cell lysis conditions are unable to release chromatin-bound proteins to the supernatant for subsequent co-immunoprecipitation assays. We utilized an endonuclease that digests chromatin DNA, which released chromatin-bound proteins in their native states, allowing efficient co-immunoprecipitation analyses (as described in Dou et al, Nature, 2015). Importantly, we detected a direct LC3-LMNB1 interaction, using purified proteins. Because LMNB1 associates with LADs, we investigated whether LC3 also binds to LADs. Indeed, we found that LC3 associates with LADs genome-wide, using LC3 chromatin immunoprecipitation and sequencing. This is the first report demonstrating an autophagy protein interacting with chromatin at the genome-wide level.

Our functional studies in primary human cells revealed that autophagy does not degrade LMNB1 upon “normal” stress, such as starvation, but specifically decomposes nuclear lamina during cellular senescence, which is induced by “aberrant” stress, such as oncogenic and genotoxic insults. The selectivity of nuclear lamina degradation has potential implications. The loss of LMNB1 causes impaired cell proliferation, disrupts lamina-associated heterochromatin, and leads to global alterations of the epigenome and transcriptome. These irreversible alterations are likely beneficial to restrain tumorigenesis, but due to the severity of the disruption, are unlikely to be a strategy to cope with starvation, a physiological condition that repetitively occurs for all living organisms.

We further dissected the LMNB1 degradation mechanism. By live-cell imaging, we observed a nucleus-to-cytoplasm shuttling process, through nuclear membrane blebbing into the cytoplasm, which is then targeted by cytoplasmic autophagy machinery. Furthermore, we mapped the LC3-LMNB1 interaction, and found that blocking this interaction impairs LMNB1 degradation and attenuates cellular senescence. Importantly, inactivating *Atg7* or expressing a LMNB1 substitution mutant deficient in LC3 binding results in accumulation of nuclear membrane blebs that are unable to bud off. These data suggest that the autophagy machinery directly controls nuclear membrane trafficking. In line with this notion, we note that the nuclear membrane blebs are reminiscent of omegasomes, the “Ω”-shaped structures on ER that are induced upon starvation and are regarded as autophagosome precursors. In senescent cells, the nuclear membrane blebs and the subsequent budding followed by digestion closely resembles the omegasome-autophagosome-autolysosome transition. We reason that the autophagy machinery may have roles in mediating trafficking of the nuclear membrane blebs to coordinate with the cytoplasmic digestion.

Our study suggests a new direction in studying mammalian autophagy from the perspective of the nucleus, and inspires many new questions, including the mechanism of nuclear membrane trafficking, the signaling events that initiate the nuclear degradation, and the general roles of autophagy proteins in the nucleus. Furthermore, while this work focuses on senescence, we speculate that the autophagic nuclear degradation may occur and have roles in other physiological and pathological conditions.