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AMBRA1-induced mitophagy: A new mechanism to cope with cancer?

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Dysfunctions in mitophagy, the process by which mitochondria are eliminated, are associated with cancer. We found that the proautophagic protein AMBRA1 (activating molecule in beclin 1 regulated autophagy) binds the autophagosome adapter LC3, and that this interaction is crucial for mitochondrial clearance with or without involvement of the E3-ligase PARKIN. The discovery of a novel mitophagy pathway has the potential to promote new anticancer strategies.

Considering that mitochondria represent the cellular powerhouses, it is not a surprise that dysfunctional mitochondria are detrimental to the cell. Accumulation of damaged mitochondria is a feature of aging and multiple types of disease, including cancer. Fortunately, mammalian cells have developed a way to remove damaged mitochondria through a form of selective autophagy called mitophagy.

Mitophagy is induced after the loss of mitochondrial membrane potential ($\Delta\Psi_m$) and mitochondrial depolarization. PTEN-induced putative kinase 1 (PINK1) is stabilized at the mitochondria, where it recruits the E3 ubiquitin ligase PARKIN. In this context, activating molecule in beclin 1 regulated autophagy (AMBRA1) interacts with PARKIN to enhance mitochondrial clearance.[1] In our study,[2] we identified AMBRA1 as a LIR (LC3-interacting region)-containing protein and found that this motif is essential for the binding between AMBRA1 and LC3 only following mitophagy induction. In line with the findings of Van Humbeeck et al.[1] we provide molecular evidence that this interaction is crucial for the promotion of PARKIN-mediated mitochondrial clearance.

In addition to the PINK1/PARKIN pathway, the so-called mitophagy receptors can also regulate mitophagy. These receptors are localized at the outer membrane of mitochondria and possess a consensus sequence (LIR motif: W/F/YxxL/I) that allows binding with LC3 for selective autophagy. To date, 2 types of mitophagy receptors have been identified: BCL2/adenovirus interacting protein 3 (best known as NIX/BNIP3L-BNIP3), which is involved in mitophagy during red blood cells maturation, and FUN14 domain containing 1 protein (best known as FUNDC1), which is involved in mitophagy induced by hypoxia or mitochondrial membrane depolarization. Our results suggest that AMBRA1 represents a third type of mitophagy receptor. We previously demonstrated that a pool of AMBRA1 could be found associated with BCL2 at the outer mitochondrial membrane.[3] We now show that AMBRA1 possesses a LIR domain that allows it to bind to LC3 upon mitophagy induction. We further demonstrate that targeting AMBRA1 to mitochondria (AMBRA1-ActA) is sufficient to induce massive mitochondrial clearance in both PARKIN-dependent and -independent systems. Interestingly, it has recently been demonstrated that BECLIN 1 (best known as BECN1) interacts with PARKIN and regulates translocation of PARKIN to the mitochondria.[4] Since we showed that downregulation of BECN1 reduces AMBRA1-ActA-induced mitophagy, it would be interesting to investigate the role of BECN1 in this process. In principle, we can hypothesize that BECN1 delivers a putative E3 ubiquitin ligase in order to ubiquitylate mitochondria in AMBRA1-ActA-induced mitophagy.

Until now it was not clear whether the major pathways of mitophagy were dependent on or independent of each other. Our work indicates that a parallel pathway for mitophagy may exist when PARKIN is not present. It should be noted that lack of PARKIN is not particularly unusual; the *PARK2* (best known as *PARKIN*) gene is silenced in a number of common cell lines, such as HeLa or mouse embryonic fibroblasts. Of note, all known mitophagy receptors are regulated by specific kinases[5,6] and it would be important to investigate whether the mitochondrial pool of AMBRA1 is subjected to post-translational modifications under mitochondrial stress.

A common feature of all mitophagic proteins is their deregulation in cancer. PARKIN-deficient mice are susceptible to hepatocarcinogenesis. In addition, the *PARK2* gene is often targeted in malignant

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tumors.[7] Together, these data define *PARK2* as a tumor suppressor gene (TSG). BNIP3 expression is lost in several cancers such as breast and pancreatic cancers[8] and NIX expression is deregulated in ductal carcinoma of the breast.[9]

In line with these data, we recently discovered that *AMBRA1* mutant mice develop spontaneous tumors. Mutations of *AMBRA1* can also be found in a number of human cancers.[10] In principle, inactivation of AMBRA1-induced mitophagy may be an aspect of the AMBRA1-deficient phenotype in tumorigenesis and warrants more detailed study as a potential prognostic indicator in cancer.

When we forced AMBRA1 to the mitochondria surface using the AMBRA1-ActA construct, we observed that all mitochondria were ubiquitylated, as also observed in PARKIN-deficient cells. Thus, we can conclude that AMBRA1-ActA most likely serves as an adaptor for E3 ligases. This would not be an unusual job for this protein. Indeed, we demonstrated that the kinase ULK1, which is important in upstream regulation of autophagy, is ubiquitylated by the E3-ligase TNF receptor associated factor 6 (TRAF6) in a manner mediated by AMBRA1.[11] It is now crucial to identify which kind of E3 ubiquitin ligase is involved in AMBRA1-induced mitophagy. To better understand the molecular mechanisms underlying the TSG function of AMBRA1, it would be interesting to identify new substrates of this unrevealed E3 ubiquitin ligase that might be related to controlling apoptosis or cell proliferation.

Another common feature of all mitophagic proteins is the negative regulation by BCL2 family members; BCL2 inhibits PARKIN-mediated mitophagy, whereas BCL2L1 inhibits FUNDC1-mediated mitophagy. We demonstrated that BCL2 could inhibit the proautophagic activity of AMBRA1[3] and it will therefore be very important to explore whether BCL2 can also control AMBRA1-induced mitophagy. Overexpression of BCL2 family members is often associated with unfavorable pathogenesis in cancer, and this might at least in part be related to their role in inhibiting the different pathways of mitophagy. In **Figure 1** we summarize our model of the role of AMBRA1 in mitophagy in mammalian cells.

In conclusion, the loss of mitophagy proteins appears to promote tumorigenesis, underlying a tumor suppressor function of mitophagy. Despite the complex nature of the role of autophagy/mitophagy in cancer, it thus appears that stimulation of mitophagy may play a pivotal role in fighting cancer. Our discovery of a new AMBRA1-induced mitophagy pathway, and in particular the identification of a new E3 ubiquitin ligase and its substrates in this context, could open new avenues of research in cancer therapy.

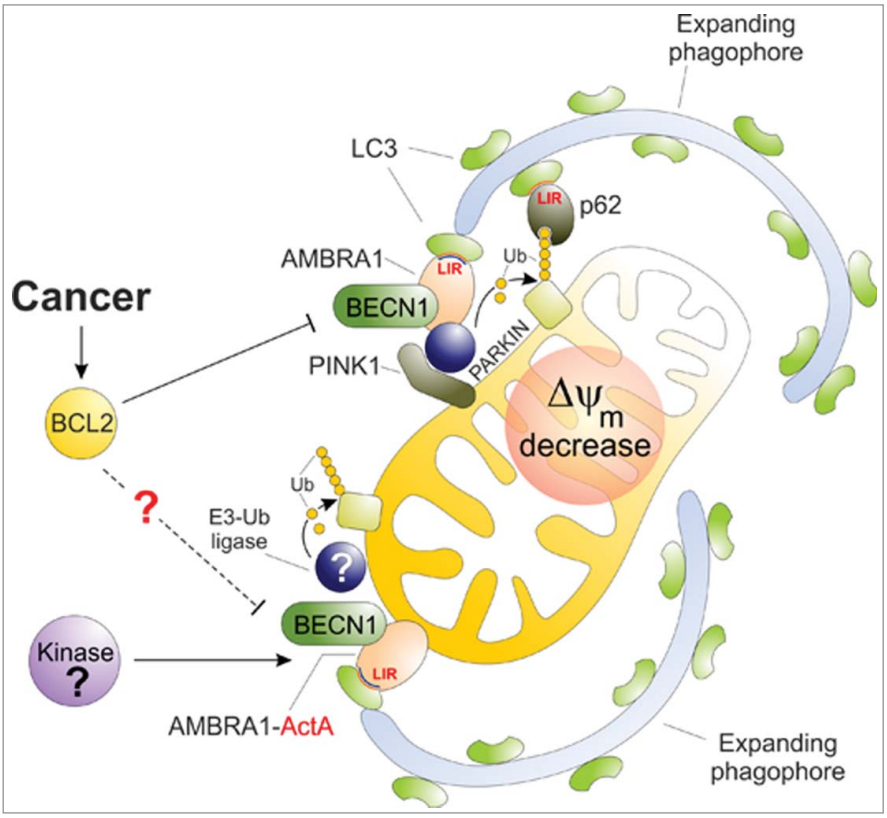

Figure 1. AMBRA1, a novel mitophagy receptor. The PINK1/PARKIN pathway is induced after mitochondrial membrane depolarization (indicated as $_{\Delta\Psi m}$ decrease in the figure). PINK1 (PTEN-induced putative kinase1) is stabilized on the mitochondria and allows mitochondrial recruitment of PARKIN. PARKIN ubiquitylates mitochondria (ubiquitin molecules are indicated as "*Ub*" in the figure), which can then be recognized by the cargo protein p62. AMBRA1 (activating molecule in beclin 1 regulated autophagy) plays a crucial role in triggering mitochondria degradation by interacting with LC3 through its LIR (LC3-interacting region) motif. Furthermore, high levels of AMBRA1-ActA at the mitochondria are able to induce massive mitophagy in both PARKIN-dependent and -independent context. It is likely that an E3 ubiquitin ligase is involved in mitochondria ubiquitylation following AMBRA1-ActA expression. Based on our knowledge of other mitophagy receptors, a kinase is probably involved in the post-translational regulation of AMBRA1 under mitochondrial stress. Finally, in cancer, an increase in BCL2 expression leads to inhibition of PARKIN-mediated mitophagy and, most likely, AMBRA1-mediated mitophagy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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