Autophagy-assisted antigen cross-presentation

Autophagosome as the argo of shared tumor-specific antigens and DAMPs

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It is generally believed that most tumor antigens are passively released from either health or dying tumor cells as intact soluble antigens, peptide fragments complexed with heat shock proteins (HSPs), or packaged in secretory vesicles in the form of microparticles or exosomes. The passive release of tumor antigens is generally non-inflammatory and non-immunogenic; however, results from others and our laboratories suggest that autophagy is critically involved in immunogenic cell death.

Autophagy is a process of packaging misfolded proteins and damaged organelles in the autophagosomes and degrading them through fusion with lysosomes. During the early development of cancer, autophagy can serve as a tumor suppression mechanism, since it clears abnormal proteins and limits genome damage. Autophagy becomes a mechanism of tumor promotion at later stages, because it helps tumor cells survive during physiological stress of metastases and stress induced by chemotherapy or irradiation.1

Notably, autophagy in cancer cells also plays a critical role by providing immunogenic tumor antigens and eliciting immune responses required for tumor cell destruction.2,3 We have recently identified macroautophagy as one of these critical pathways that regulates antigen delivery, and shown that tumor macroautophagy regulates the efficiency of cross-presentation.2 Tumor cells that undergo autophagy release autophagosomes, which are important tumor antigen sources for cross-priming of tumor-specific CD8+ T cells, into the culture media.3 By inducing autophagy and blocking protein degradation through proteasome and lysosome inhibition, we demonstrated that large amounts of secreted autophagosomes contain abundant ubiquitinated antigens. Using OVA and gp100 as model antigens, we showed that the tumor antigens sequestered in autophagosomes were efficiently cross-presented to naive transgenic OT-I and pmel-1 CD8+ T cells respectively in vitro and in vivo. Compared with soluble antigens, antigens packaged in the autophagosomes were superior in activating CD8+ T cells.

We then investigated the mechanisms by which antigens in autophagosomes were cross-presented by DCs. CLEC9A, a novel C-type selectin receptor on a subset of DCs, plays an important role in cross-presentation of dead cell-associated antigens. Interestingly, we detected high expression of CLEC9A ligand on autophagosomes. Blockade of the interaction between the CLEC9A ligand on autophagosomes and CLEC9A on DCs significantly reduced antigen cross-presentation, but did not affect uptake of autophagosomes by DCs. CLEC9A is likely involved in the intracellular process rather than phagocytosis of autophagosomes. Moreover, the size of autophagosomes (200–900 nm) may obligate them to non-acidic intracellular compartments that favor MHC I presentation on DCs. Utilizing specific inhibitors of distinct types of endocytosis, we showed that cross-presentation of autophagosome derived antigens depended predominantly on the caveolae-mediated endocytosis pathway, which routes antigens to non-acidic compartments. We further demonstrated that tumor antigens present in the autophagosome must exit through the ERAD translocation machinery after which they are degraded by pAPC proteosomes. Thus, the autophagy-assisted antigen cross presentation pathway also requires the phagosome-ER-cytosol route of cross-presentation.4

These unique characteristics of tumor-derived autophagosomes make them an ideal vaccine candidate for cancer immunotherapy. Using the 3LL Lewis lung cancer and B16F10 melanoma models, we showed that tumor-derived autophagosomes loaded onto DCs combined with poly I:C as the adjuvant significantly delayed tumor growth or eradicated tumors in C57BL/6 mice bearing established 3LL lung tumors. We also found potent therapeutic activity in mice with orthotopically transplanted primary...
Many chemotherapy drugs and irradiation induce autophagy while killing the tumor cells. Our findings indicate that autophagosomes play an important role as antigen carriers, which suggests that cells dying with autophagy may release autophagosome antigens and inflammation signals into the local environments or body fluids and elicit a strong CD8+ T-cell immune responses. Interestingly, Kroemer's group recently discovered that autophagy provides energy required for priming of anti-tumor T-cell immunity by the release of ATG from dying cells. Thus, autophagy in dying tumor cells plays a critical role in induction of anti-tumor immune response through multiple mechanisms. Our group is exploiting these mechanisms and evaluating the effectiveness of autophagosomes from cancer cells as vaccines for patients with cancer.

**Figure 1.** Autophagy-assisted antigen cross presentation and immunogenic cell death. (A) Tumor cells dying with autophagy produce autophagosomes that contain a variety of tumor antigens, activators of the innate immune system, and molecules that target autophagosomes to DCs. (B) Dying tumor cells release HMGB1 and other alarmins that mobilize and activate DCs. (C) Tumor cells dying with autophagy produce ATP and other molecules that directly or indirectly acting on T cells to enhance their activation, proliferation and effector functions.
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