Tricking Melanoma to Self-Digest: A Deal of a Meal!

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Melanoma cells acquire multiple genetic and epigenetic alterations that promote their metastasis and resistance to available therapies. In this issue of *Cancer Cell*, Soengas and colleagues reveal that the induction of endosome-mediated autophagy results in efficient melanoma cell death, thereby offering new potential means for treatment of this devastating cancer.

Advanced melanoma often presents itself as a metastatic disease highly resistant to available therapies. Advances made over the past decades have revealed the genetic landscape of melanoma formation, including mutations in *NRAS*, *BRAF*, and related signaling pathways. Often, resistance of melanoma to chemotherapy is attributed to modification of cell-death programs (Ivanov et al., 2003). The work of Soengas and colleagues reveals a novel approach that we may be able to exploit to eliminate this devastating cancer (Tormo et al., 2009). The study describes the induction of autodigestion of melanoma cells in culture and in mouse melanoma models by a dsRNA mimic combined with a specific delivery vehicle polyethyleneimine (PEI). This is a meal we all will cherish.

Autodigestion of melanoma cells may sound like science fiction; nonetheless, it appears to utilize a well-conserved, self-eating pathway through which cellular components are eliminated under stress conditions. This process, known as autophagy, serves to recycle macromolecules and organelles to maintain cellular homeostasis. Autophagy has a cytoprotective function by protecting cells from potentially lethal, stressful conditions, including metabolic stress and infection. Recently, we have come to recognize that dysregulation of autophagy is closely associated with neurodegenerative and infectious diseases, as well as cancer (Levine and Kroemer, 2008). Correspondingly, growing evidence supports a role for autophagy in cancer pathophysiology (Eisenberg-Lerner and Kimchi, 2009). Extensive crosstalk exists between autophagy and cell death (Maiuri et al., 2007), although the factors that govern this crosstalk remain elusive. The present work demonstrates that changes in the link between these processes in melanoma can be exploited to convert cytoprotection to cytokilling. Accordingly, manipulating autophagy may offer a useful approach to cancer treatment. Along these lines is the example of renal cell carcinoma, where small molecules regulating autophagy/cell death offer a novel treatment opportunity (Turcotte et al., 2008).

In a search for agents that induce autophagy accompanied by melanoma tumor cell death, Tormo and colleagues showed that [pIC] (poly(dI:dC)), a classical dsRNA mimic, could elicit powerful but selective induction of melanoma cell death when combined with the carrier PEI $[pIC]^{PEI}$. The authors use a combination of various approaches to dissect the mechanism of action of $[pIC]^{PEI}$ and demonstrate that $[pIC]^{PEI}$-mediated death occurs by an active endosome-autophagosome-lysosome fusion, namely endosome-mediated autophagy. Accordingly, mouse embryo fibroblasts lacking a key gene required for autophagy, *Atg5*, showed a marked decrease in $[pIC]^{PEI}$-induced autophagy and cell death. While melanoma cells have been extensively studied for changes in classical apoptotic programs, the mechanisms underlying their basal and drug-induced autophagy are largely unexplored. Therefore, the finding that it is possible to induce melanoma cells' death by inducing autophagy is novel and somewhat surprising. Furthermore, given the notion that tumor cells adapt to high nutritional stress (Levine and Kroemer, 2008), the treatment identified by Tormo et al. could bypass multiple barriers. The authors suggest that the ability of $[pIC]^{PEI}$ to trigger multiple fusion cycles is a key for converting cytoprotective autophagy to one that triggers efficient death of melanoma cells.

Mechanistically, this study identified melanoma-differentiation-associated gene 5 (MDA-5) as a key component required to link autophagy to melanoma cell death following $[pIC]^{PEI}$ treatment. MDA-5 is a dsRNA helicase implicated in interferon-mediated innate immune responses (Kato et al., 2006). Yet the Soengas group show that the $[pIC]^{PEI}$ complex can engage MDA-5 and promote tumor cell death even in highly immunosuppressed mice. Recruitment of the small GTPase Rab7 and lysosomal activity were also identified by the authors as important for persistent cycles of fusion events. Although autophagy in innate immunity is normally cytoprotective, the authors revealed that components of the classic autophagy pathway are required for concomitant induction of cell death in response to $[pIC]^{PEI}$ treatment. As immune tolerance is a defining characteristic of melanoma tumors (Kirkwood et al., 2008), these results could provide an alternative to melanoma therapy.

Intriguingly, cell death reported following $[pIC]^{PEI}$ treatment took place several hours after initiation of autophagy. Among the components required for efficient tumor cell demise were NOXA and caspases, which are bona fide cell death inducers. The link between autophagy and cell death was further substantiated by demonstrating MDA5-dependent activation of NOXA and that $[pIC]^{PEI}$-induced autophagy/cell death of melanoma cells required both MDA5 and NOXA. A possible explanation for the link between autophagy and cell-death induction is provided by the sustained waves of endosome generation/maturation/resolution (Figure 1), which may lower the threshold for activation of death programs, although support for this hypothesis, as well as the nature of the delayed response, awaits further investigation.

The possible therapeutic significance of these findings is illustrated by the use of

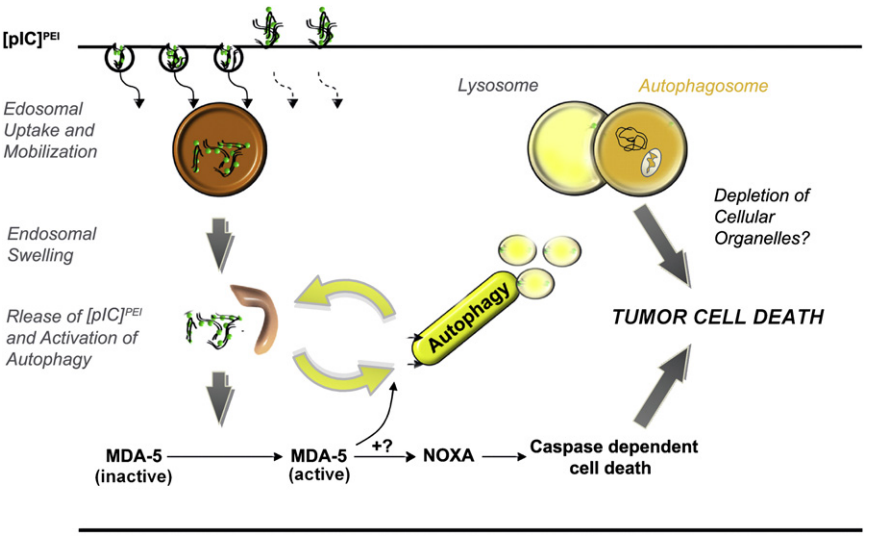

Figure 1. Proposed Mechanisms of [pIC]^{PEI}-Mediated Melanoma Cell Death

[pIC]^{PEI}-mediated tumor cell death can be achieved via two pathways. First, [pIC]^{PEI} uptake induces multiple endosomal mobilization followed by release to cytosol and activation of autophagy. Multiple sustained waves of endosome/autophagosome/lysosomal fusion events may cause depletion of vital cellular components (that maintains threshold of viability), followed by cell death or sensitization of cells to subsequent activation of cell-death machinery. Second, activation of MDA-5 links to NOXA expression and caspase-dependent cell death, which can be facilitated by the first mechanism. Overall, two pathways (possibly interlinked) potentiate melanoma cell death.

a genetically engineered mouse melanoma model (*Tyr::NRas^{Q61K}/Ink4a/Arf$^{-/-}$*) in addition to xenografts of melanoma cells in both immune-competent and -deficient mice. In all systems, [pIC]^{PEI} efficiently reduced melanoma tumor burden with a corresponding decrease in metastasis. The reduced sensitivity of MDA-5-deficient transformed MEFs to [pIC]^{PEI} in the xenograft model compared with MDA-5-expressing cells further supports a central role for autophagy in programmed death of these tumor cells.

These findings raise several questions: among them is the selectivity of [pIC]^{PEI} toward melanoma, but not melanocytes or fibroblasts. Understanding the basis of such selectivity would not only significantly contribute to understanding how melanoma cells respond to various therapies, but also indicate if normal cell types could be inadvertently targeted. Another question relates to the use of PEI. Naked pIC was found to signal just transiently in melanoma cells, likely as a result of its degradation in the cytosol or negative feedback that blunts antiviral responses (Stetson and Medzhitov, 2006). As PEI enhances delivery of nucleic acids to the cytosol (Akinc, et al., 2005), it is plausible that [pIC]^{PEI} might escape the degradative pathway, leading to sustained autophagy and MDA-5 activation (Figure 1). Further studies could determine the underlying mechanism and perhaps identify other compounds of similar or greater efficacy and selectivity. Lastly, it is of equal importance to assess the potential for development of resistance to this treatment. Also of interest is to elucidate the regulation of endosomal dynamics and its putative contribution to cell death, which are largely unexplored and are relevant for melanosome biogenesis and melanoma biology.

Taken together, this work offers new insight into the induction of endosome-mediated autophagy as a means to promote melanoma cell death. It also sheds light on the use of dsRNA as a therapeutic strategy and the role of MDA5 and NOXA in endosomal maturation linked to autophagic death. Given the failure rate in melanoma therapy and the fact that most melanoma therapies have utilized rather traditional approaches, this work offers new thoughts on potential treatment of this devastating cancer.

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