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When cytoprotective autophagy isn't… and even when it is

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Multiple papers have been published that have identified and/or characterized the cytoprotective function of autophagy, primarily in tumor cells exposed to chemotherapy or radiation. These studies have relied on pharmacological and/or genetic interference with autophagy to establish its protective function, often primarily by demonstrating that cells in which autophagy has been suppressed undergo increased apoptosis. The purpose of this Editor's Corner is to emphasize that these approaches, while absolutely necessary, are of themselves insufficient to support the conclusion that autophagy is cytoprotective in a given experimental tumor line exposed to a particular agent; complementary studies are required that demonstrate that autophagy inhibition sensitizes the tumor cell to the autophagy-inducing treatment. Otherwise, autophagy may be responsible for the growth arrest and/or cell death that is observed with the drug or radiation treatment alone, and autophagy inhibition may simply be converting one form of growth inhibition/cell death to an alternative pathway that achieves the same end result in terms of sensitivity to the treatment.

It has long been known that a primary function of autophagy is that of cytoprotection, characterized by the self-cannibalization of cellular organelles under conditions of nutrient deprivation, thereby allowing the cell to survive, albeit in a nonproliferative state. This arrested state is ostensibly reversed when the environmental conditions become favorable for further growth and ultimate cellular division. It therefore has been logical to assume that autophagy induced by chemotherapy and/or radiation in tumor cells would likely serve a similar cytoprotective function. Presumably this function of autophagy would not involve the degradation of cellular materials to provide nutrients and metabolic precursors since the cell is not experiencing a material deficiency in metabolic materials. Instead, the putative cytoprotective function of autophagy under conditions of external forms of stress imposed by chemotherapeutic drugs or radiation is generally considered to reflect the capacity of the cell to eliminate toxic species such as free radicals and possibly damaged and misfolded proteins or organelles. In this context, a question that yet remains to be resolved is how autophagy can serve cytoprotective functions in response to agents that have fundamentally different mechanisms of action.

The cytoprotective form of autophagy has almost uniformly become associated with the evasion of apoptotic cell death, as apoptosis is frequently observed when chemotherapy- or radiation-induced autophagy is inhibited. This induction of apoptosis may be related, in large part, to the crosstalk between the autophagic and apoptotic signaling pathways[1] and the consequent capacity of autophagy to prevent apoptosis. Given the extensive literature that has based the premise of autophagy serving a cytoprotective function on the observations that pharmacological and/or genetic suppression of autophagy promotes apoptosis, it is incumbent upon those of us working in the field to recognize that these experimental approaches, while of absolute necessity, are insufficient to support this conclusion. This is a consequence of the fact that the autophagy induced by cancer chemotherapeutic drugs or radiation may of itself be attenuating cellular proliferation and/or promoting tumor cell death in the absence of apoptosis, necrosis, necroptosis, or mitotic catastrophe. That is, the tumor cells are often shown to be relatively sensitive to the autophagy-promoting stress. In order to establish that autophagy is actually serving a cytoprotective function, it is necessary to demonstrate that autophagy inhibition by both pharmacological and genetic approaches results in a clear and unequivocal increase in sensitivity to the autophagy-inducing external stress based on rigorous and unequivocal assays such as clonogenic survival. In the absence of such data, or if drug/radiation sensitivity is unaltered when autophagy is inhibited, then autophagy may of itself be serving to mediate the antitumor actions of the drugs or radiation, or may have no functional relevance to these outcomes (which we have previously termed nonprotective autophagy).[2] The fact that apoptosis is frequently induced when autophagy is inhibited likely reflects the irreversible commitment of the cell to die in the face of an externally imposed stress. Blocking one possibly preferred pathway that can lead toward cell death, such as autophagy, can be considered somewhat analogous to an impediment in the path of a boulder rolling headlong down a mountain. Encountering the impediment does not prevent the boulder from reaching its inevitable resting place at the base of the mountain, but simply deflects it onto an alternative course. Consequently, autophagy and apoptosis could in many cases

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reflect alternative pathways that steer the cell toward the identical endpoint (presumably similar to the case of a cell that is incapable of undergoing apoptosis instead dying through autophagy in the face of an externally induced stress).[1]

In the context of determining the type(s) of experimental data that should be utilized in concluding that autophagy in a particular experimental model is cytoprotective in function, it is necessary to emphasize that experiments in which autophagy is inhibited by pharmacological approaches alone are clearly insufficient for this purpose. Aside from the possibility of off-target or alternative target effects of the pharmacological inhibitors (generally chloroquine, bafilomycin A_1, 3-methyladenine, or ammonium chloride),[3] the toxic or antiproliferative effects of these agents alone frequently fail to be factored into the equation. That is, when chemotherapy or radiation is combined with the pharmacological autophagy inhibitor, the impact on viable cell number is often simply additive. Since the combination treatment produces a more pronounced effect than the drug or radiation alone, there is a tendency to interpret the outcome as reflecting interference with the cytoprotective function of autophagy. While this may sometimes be the case, it is necessary to also consider that what may be occurring is conversion of the cell death or reduced self-renewal capacity induced by autophagy to a cell death/reduced self-renewal capacity induced through apoptosis coupled with the toxicity/antiproliferative effects of the autophagy inhibitor. This is generally not a problem with genetic silencing of autophagy regulatory genes except in such cases where silencing itself might be growth suppressive.

This is not to argue against the existence and potential therapeutic utility of harnessing the cytoprotective functions of autophagy to sensitize tumor cells to therapy through autophagy inhibition. Rather it is to argue for the enforcement of rigorous experimental guidelines in order to identify the specific function(s) of autophagy in a particular experimental model system. An example of a paper where cytoprotective autophagy appears to have been established by rigorous and complementary experimental approaches is the study by Soto-Pantoja et al. where pharmacological and genetic inhibition of autophagy was shown to sensitize T cells and endothelial cells lacking CD47 to radiation.[4]

Although autophagy can unequivocally be proven to have a cytoprotective function in some experimental models, the concept that autophagy induction represents a generalized mechanism of tumor resistance to therapy may prove to be overly broad and all encompassing. Autophagy can be shown to have multiple functions in addition to being cytoprotective, with additional outcomes being cytotoxic, nonprotective, and cytostatic.[5] As only one of these functions has the potential to be exploited for therapeutic advantage, a more nuanced view of the cytoprotective form of autophagy as a therapeutic target is likely to be appropriate.

Finally, assuming that the experimental foundation for cytoprotective autophagy in a particular model/cell culture system (radiation/drug and tumor) is firmly established, such findings should ideally be supported by studies in tumor-bearing animals. Furthermore, it is important to recognize that Kroemer's group has shown that efforts to restrain tumor cell autophagy in vivo are likely to simultaneously interfere with the generation of danger/eat-me signals that are necessary for the effective engagement of immune responses that may be required for successful tumor elimination.[6] Consequently, the many studies in nonsyngeneic xenograft model systems could be providing an incomplete (and possibly misleading) picture of the potential effectiveness of autophagy inhibition as a therapeutic strategy. Efforts to exploit the cytoprotective functions of autophagy for therapeutic benefit would likely best be served by studies of tumor suppression and, perhaps even more critically, prolongation of survival in immune competent tumor-bearing experimental models.

Disclosure of Potential Conflicts of Interest

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