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REVIEW

The Autophagic Response to Radiation: Relevance for Radiation Sensitization in Cancer Therapy

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Radiation almost uniformly promotes autophagy in tumor cells. While radiation-induced autophagy often serves as a protective function in cell culture studies, it is currently uncertain to what extent autophagy might be induced by radiation in human malignancies; it is furthermore unknown whether autophagy induced by radiation can or should be suppressed for therapeutic benefit. Current clinical trials combining chemotherapeutic drugs or radiation therapy with chloroquine or hydroxychloroquine as autophagy inhibitors may be premature without the benefit of stratification to identify patients whose malignancies might be susceptible to autophagy inhibition as a therapeutic strategy. In addition, there are also concerns as to whether chloroquine and hydroxychloroquine, the agents currently in use, have the capacity to suppress autophagy when administered systemically at tolerable doses. Finally, any agent that actually has the appropriate pharmacokinetic profile to function as a systemic autophagy inhibitor may collaterally disrupt the homeostatic function of autophagy in normal cells. **© 2014 by Radiation Research Society**

Virtually every study in the literature has found that clinically relevant doses of radiation promote autophagy in tumor cells (*1–10*). Figure 1 shows three assays indicative of radiation-induced autophagy in tumor cells, in this case the H460 non-small cell lung cancer cell line. Given the near-universality of this response, and the extensive literature supporting the cytoprotective functions of chemotherapy-induced autophagy (*11–13*), there has been a tendency to conclude that radiation-induced autophagy is also, by its nature, a cytoprotective response, one that presumably has a role in conferring resistance to radiation therapy (*2–8*). However, this is unlikely to actually be the case, since autophagy is induced across a spectrum of tumor cell lines and there is no evidence that autophagy induction is limited to tumor cells that might be considered to be radiation resistant. Nevertheless, it may prove feasible to exploit radiation-induced autophagy for therapeutic benefit against those tumors where autophagy is found to have a cytoprotective function (i.e., where inhibition of autophagy results in an improved response to radiation) whether or not the tumor is considered to be radiation ''sensitive'' or ''resistant''. Consequently, one of the primary purposes of this review is to discuss whether autophagy inhibition, as a strategy for improving the response to radiation therapy, has a reasonably sound experimental foundation.

A closely related question, in the event that autophagy inhibition *can* be determined to consistently radiosensitize tumor cells is whether the extent of radiosensitization that may occur with inhibition of radiation-induced autophagy is of sufficient extent and intensity to justify taking this strategy into clinical trials combining autophagy inhibition with radiation therapy. In this context, cell culture studies alone are clearly insufficient and increased efficacy would have to be demonstrable in tumor-bearing animal models. Ideally, in addition to such standard end points as tumor growth delay, it would also be critical to demonstrate a significant prolongation of animal survival over and above that produced by radiation treatment alone (*14*). Here, as in all animal-based studies, we are challenged by the choice of appropriate and relevant animal models. In particular, tumor xenografts may be inappropriate since it has been postulated, based on rigorous experimental data, that the immune system is likely to play a central role in contributing to the effectiveness of cancer chemotherapeutic drugs and radiation therapy (*8, 15*). Specifically, it appears that suppression of autophagy is likely to interfere with the capacity of the immune system to facilitate tumor elimination.

We would argue, based primarily on the literature relating to inhibition of chemotherapy-induced autophagy, that in many studies, the extent of sensitization by chloroquine or hydroxychloroquine (the drugs routinely used as autophagy inhibitors in animal studies as well as in ongoing clinical

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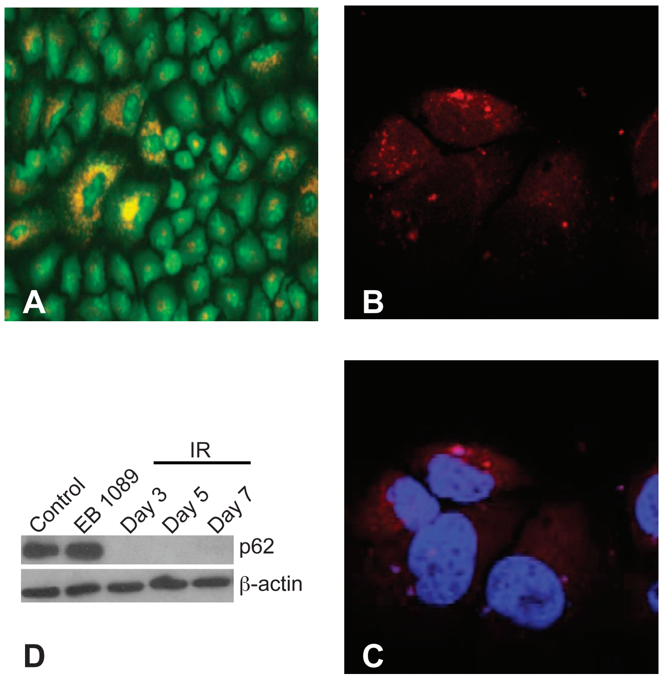

FIG. 1. H460 non-small cell lung cancer cells were exposed to a radiation dose of 6 Gy, a dose that does not promote apoptosis. Panel A: Acridine orange staining of autophagic vesicle formation. Panel B: RFP-LC3 transfected cells showing autophagic vesicle formation. Panel C: Co-staining with DAPI and RFP-LC3 to demonstrate that autophagic vesicle formation is largely extranuclear. Panel D: Western blotting demonstrating the degradation of p62 after radiation (IR) exposure, which is indicative of autophagic flux (and lack of effect on autophagic flux of the vitamin D analog, EB 1089).

trials) is relatively modest (*14*). Although these combination treatment approaches often result in a prolongation of tumor growth delay, they rarely demonstrate tumor cell killing and rarely if ever demonstrate prolonged survival of tumor-bearing mice compare to the therapy alone (*14*). This lack of tumor cell killing could be a critical deficiency in efforts to sensitize malignancies through autophagy inhibition since the cytoprotective function of autophagy is often identified based on the promotion of apoptotic cell death when autophagy is inhibited in tumor cells in culture (*16*).

The overall function of autophagy, as currently understood, is to eliminate misfolded proteins and damaged organelles as well as to suppress potentially injurious reactive oxygen species (*17*). Historically, autophagy has facilitated cell survival under conditions of nutrient deficiency by generating nutrients and metabolic precursors from the degradation of cellular organelles through the fusion of autophagic vesicles enclosing these organelles with hydrolase-containing lysosomes (*17–20*). Autophagy also appears to have dual and conflicting functions in oncogenesis. Autophagy can initially prevent or at least delay tumor formation by protecting the cell from potentially damaging species that might lead to mutational and carcinogenic damage, however, once tumor formation has progressed, autophagy can protect the tumor cell from environmental injury (*21, 22*). In radiation therapy (and chemotherapy), the induction of autophagy is frequently thought to perform an additional cytoprotective function by preventing cell death through apoptosis, which may occur in part through the extensive and likely elaborate crosstalk between autophagic and apoptotic signaling pathways (*23, 24*). In addition, there is accumulating evidence that autophagy can promote or accelerate senescence (*25*). We and others have reported that senescence is a primary response to radiation exposure (*26*), but whether senescence serves a cytoprotective function by facilitating long-term cellular survival or is a precursor to one or more forms of cell death is still subject to debate (*27*). To add another level of complexity to this issue, it has been argued that, as with autophagy, senescent cells also activate an immune recognition response that contributes to the elimination of the tumor cell (*28*).

As indicated above and shown by our laboratory as well as by others, ionizing radiation frequently promotes a cytoprotective form of autophagy (*2–8*). Proof of function is established by the observation that radiation sensitivity is increased when autophagy is inhibited either pharmacologically or genetically and that autophagy inhibition further promotes apoptotic cell death. This has been shown quite unequivocally in breast tumor cell lines such as MCF-7 and ZR-75 (*6, 7*) and in H460 and A549 non-small cell lung cancer cells (*8*). However, we have also reported that in breast tumor cells, 4T1 and Hs578t (*14*), and more recently in HN6 head and neck cancer cells and H838 non-small cell lung cancer cells (unpublished results), inhibition of autophagy neither sensitizes nor protects the tumor cells from radiation. We have termed this form of autophagy ''nonprotective'' (*14, 29*).

In studies where autophagy has been found to exhibit a cytotoxic function, these have almost uniformly involved radiation in combination with a radiosensitizing agent (*30–35*), here it should be emphasized that the capacity of autophagy to directly mediate cell death remains controversial. Clearly, autophagy inhibition would likely attenuate the impact of radiation under these conditions, assuming preclinical studies are predictive of clinical outcomes. In our own work in breast cancer cells, vitamin D or vitamin D analogs have been shown to promote cell death through autophagy in MCF-7 and ZR-75 breast tumor cells, two of the same cell lines in which radiation alone promotes cytoprotective autophagy (*6, 7*).

In recent efforts to extend our findings with vitamin D to non-small cell lung cancer cells, where radiation sensitization would likely have a much greater clinical impact than in breast cancer because of the limited effectiveness of therapy in prolonging the lifespan of these patients, we have also observed a switch from cytoprotective autophagy to a form of autophagy that enhances radiation sensitivity (in clonogenic survival assays) without providing direct evidence of cell killing, which we have termed cytostatic

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TABLE 1
Impact of Autophagy Inhibition on Radiation Sensitivity for the Four Different Forms/Functions of Radiation-Induced Autophagy (*29*)

Autophagy function	External stress	Impact of autophagy inhibition
Cytoprotective	Radiation alone	Radiation-induced tumor cell death; radiation sensitivity enhanced (*2–8*)
Cytotoxic	Radiation + modulator	Tumor cell survival; radiation sensitivity attenuated (*6, 7, 30–33*)
Nonprotective	Radiation alone	Tumor cell sensitivity to radiation unaltered (*14*)
Cytostatic	Radiation + modulator	Tumor growth resumes; radiation sensitivity attenuated[2]

autophagy (*29*).[2] We do recognize that growth arrest in the context of autophagy induction actually occurs in the case of nutrient deprivation (*18, 19*). However, to our knowledge this cytostatic form of autophagy has never previously been associated with sensitization to radiation (or chemotherapy).

As indicated earlier in this review, the importance of recognizing and distinguishing between the different forms of (radiation-induced) autophagy relates to the potential for increasing sensitivity of tumor cells to radiation through inhibition of the cytoprotective form of autophagy. However, this possibility is based on the presumption that the form of autophagy induced by clinically relevant doses of radiation in a patient's tumor is actually cytoprotective. One fundamental problem with this strategy is that there is not, as yet, conclusive proof that radiation therapy promotes autophagy in patient tumors of any origin. Furthermore, even if we assume that radiation therapy does induce autophagy in (some, if perhaps not all) clinical malignancies, there is no assurance that autophagy will have a cytoprotective form and function. This issue is made all the more difficult and challenging by the fact that we have no uniformly established and validated protocol for detecting autophagy in clinical samples [assuming that early biopsies are accessible and approval for their access is obtained from the appropriate Institutional Review Boards (IRBs)]. Finally, even if and when autophagy induction can be conclusively determined to occur in patient tumors, identification of the form and function of that autophagy is beyond the reach of current assay technologies. In fact, to our knowledge there is little or no information that could distinguish the putative different forms of autophagy based on biochemical, molecular or morphological characteristics, even in cell culture systems (*29*). The data in Table 1 show the potential impact of interfering with the four different functional forms of autophagy induced by radiation alone and by radiation in combination with radiation sensitizers.

Two additional factors should also be considered in the process of deciding whether autophagy inhibition might prove to be useful in efforts to enhance tumor cell sensitivity to radiation. There are few studies of autophagy induction by radiation therapy in normal cells and insufficient consideration of the possibility that systemic interference with autophagy might be detrimental to normal tissue. This could be a significant issue in terms of vulnerability of the central nervous system where defective autophagy has been associated with a number of neurodegenerative diseases (*36*). Furthermore, it is critical to consider whether direct autophagy inhibition utilizing chloroquine/hydroxychloroquine is likely to be an effective therapeutic strategy when taking into account the clinical pharmacokinetics of these agents and their putative capacity to suppress autophagy in the clinic (*14*). In this context, new autophagy inhibitors that are anticipated to have superior pharmacokinetic properties as well as clinical efficacy are currently under development (*37*).

In conclusion, while radiation quite consistently induces autophagy in tumor cells, and while the radiation-induced autophagy generally tends to be cytoprotective, the extent of sensitization that can be induced by pharmacological or genetic inhibition of autophagy varies over an extensive range and there is little if any data in tumor-bearing animals that might support clinical trials. When administered in combination with various radiosensitizing agents, radiation-induced autophagy may take different forms leading to prolonged growth arrest (cytostatic autophagy) or cell death (cytotoxic autophagy). However, there is little certainty that: 1. Autophagy is induced in a patient's tumor when radiation is clinically administered in a conventional fashion; 2. Autophagy putatively induced by radiation therapy will have a cytoprotective function in patient tumors; 3. Systemically administered agents (such as hydroxychloroquine) can achieve concentrations in the circulation that will effectively interfere with autophagy in the tumor cell; or 4. Such inhibition will produce alterations in radiation sensitivity sufficient to significantly influence tumor growth or prolong patient survival. Finally, there is insufficient data to provide assurance that systemic autophagy inhibitors will not interfere with autophagy functions in normal cells, functions that might be critical to their survival.

Given these caveats, we might be inclined to argue that clinical trials of chloroquine/hydroxychloroquine in combination with radiation therapy would be premature since preclinical data has not provided sufficient proof of principle to support such efforts. However, it might likewise be premature to entirely abandon this therapeutic

[2] Sharma K, Goehe RW, Di X, Torti S, Torti F, Gewirtz DA. A novel cytostatic form of autophagy in sensitization of non-small cell lung cancer cells to radiation by vitamin D and the vitamin D analog, EB 1089. (Manuscript submitted for publication.)

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approach in the absence of rigorous data to establish whether radiation therapy does, in fact, promote a cytoprotective form of autophagy in particular malignancies. Appropriate clinical trials should likely await the development of drugs that might achieve therapeutically effective inhibition of autophagy in tumor cells without compromising the homeostatic functions of autophagy in normal cells. The autophagy field is still in relative infancy and may hold promise if: 1. The preclinical studies can be held to rigorous standards and unequivocal end points; 2. Clinical trials could be delayed until a clearer picture develops in terms of the profile of patients whose tumors might be susceptible to autophagy inhibition as a therapeutic strategy; 3. Autophagy inhibitors with appropriate systemic and cellular pharmacokinetic properties could be developed; and 4. We could begin to understand the extent to which cytoprotective autophagy would have to be inhibited to have a significant impact in prolonging patient survival.

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