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The challenge of developing autophagy inhibition as a therapeutic strategy

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Abstract

The finding that cancer chemotherapeutic drugs and ionizing radiation often promote autophagy has provided the foundation for clinical trials combining autophagy-blocking agents with antitumor drugs and radiation. The premise driving these trials is that therapy-induced autophagy is cytoprotective; consequently, inhibition of autophagy is anticipated to sensitize malignancies to therapy. However, in it is well-established that autophagy may also mediate the toxicity of antitumor drugs while evidence also exists for a nonprotective function of autophagy. Consequently, given that it cannot be predicted what form autophagy will take upon treatment with chemotherapy or radiation, the current ongoing clinical trials are likely to generate contradictory or inconsistent results, with the potential consequence that autophagy inhibition could be dismissed as therapeutic strategy based on what are essentially false negative outcomes. Appropriate interpretation of the outcomes of these trials would require knowledge as to whether the drugs or radiation utilized promote the cytoprotective form of autophagy in the tumor cells as well as whether the chloroquine or hydroxychloroquine actually inhibited the autophagy. Ultimately, it will be necessary to identify those patients for whom the strategy of autophagy inhibition would be anticipated to improve the response to therapy. However, this is currently not feasible in the absence of appropriate bioassays or predictive markers for characterization of the autophagy or the effectiveness of pharmacological approaches for autophagy inhibition in the clinic.

Cytoprotective Autophagy in Cancer Therapy

It has long been recognized that the degradation of subcellular organelles through the process of autophagy provides energy and metabolic precursors necessary to sustain cell survival under conditions of hypoxia or nutrient deprivation (1). The concept that autophagy can also be considered a “first responder” to various other forms of stress, specifically those provoked by cancer chemotherapeutic drugs and radiation, is supported by studies in a variety of tumor cell models exposed to agents from multiple drug classes (2–6). While many of these therapeutic modalities are clearly designed to be toxic to the tumor cell, direct survival advantages that autophagy might confer remain obscure since, with some

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exceptions, chemotherapeutic drugs and radiation generally are not considered to deprive the tumor cell of its metabolic and nutritional support. Nevertheless, the autophagic response to cancer therapeutics is frequently cytoprotective in function; specifically, inhibition of chemotherapy and radiation induced autophagy by either pharmacological agents or genetic manipulation often results in a reduction in tumor cell survival if not enhanced tumor cell killing (2–13). However, as discussed in some detail below, autophagy is not always cytoprotective. Furthermore, inhibition of autophagy is likely to influence the immune response to therapy, the tumor stroma and normal tissue function.

Cytoprotective and Cytototoxic Autophagy in Cancer Therapy

Evidence that chemotherapeutic drugs and radiation promote *cytoprotective* autophagy is often based upon the observation that apoptosis is increased when the autophagy is inhibited through pharmacological or genetic approaches. An increase in the extent of apoptosis supports the premise that autophagy has the capacity to interfere with induction of the apoptotic response pathway, and there is considerable evidence for crosstalk between autophagy and apoptosis (14, 15). What is frequently overlooked in many studies is that an increase in apoptosis is not necessarily or uniformly accompanied by an enhancement of drug or radiation sensitivity (16). That is, these studies may fail to demonstrate that the combined treatment with chemotherapy or radiation and pharmacological or genetic autophagy inhibition results in a more pronounced antitumor response based on e.g. a simple assessment of viable cell number by such common methods as trypan blue exclusion, the release of lactate dehydrogenase as an indication of cell death or compromised clonogenic survival (i.e. reproductive cell death), which is generally considered the “gold standard” measurement of drug or radiation sensitivity. In fact, the autophagy induced by the therapeutic agent(s) may actually prove to be largely *cytotoxic*, where the autophagy itself is mediating drug or radiation killing in the tumor cell; consequently the mode of cell death is merely being switched from autophagy to apoptosis. In this context, there is extensive evidence for the cytotoxic function of autophagy that is expressed in a host of tumor cell models and in response to a spectrum of therapeutic insults to the tumor cell (5). Interestingly and perhaps unexpectedly, aside from the functional distinction based on the consequences of autophagy inhibition, there is no unequivocal evidence to indicate that *cytotoxic* autophagy has biochemical or molecular characteristics that would distinguish it from the *cytoprotective* form. Although it would appear intuitive to expect that cytotoxic autophagy would reflect unrestrained and excessive degradation of cellular components, a form of self-cannibalism that would ultimately compromise cell survival, this has not actually been shown to be the case.

Clinical Trials

Given the fact that autophagy may express either cytoprotective or cytotoxic function, it is predictable that ongoing clinical trials involving the combination of the pharmacological autophagy inhibitors chloroquine or hydroxychloroquine with chemotherapy or radiation in various forms of cancer would likely generate contradictory or at the very least equivocal results (17). That is, in those cases where the therapeutic agent promotes cytoprotective autophagy in patient tumors, autophagy inhibition should theoretically enhance tumor cell

sensitivity to the radiation or the drugs inducing the autophagic response. Conversely, where autophagy is initially cytotoxic, autophagy inhibition might be anticipated to interfere with the effectiveness of therapy. Alternatively, autophagy that is initially cytotoxic might be converted to an alternative form of cell death such as apoptosis, with the consequence that drug/radiation sensitivity would essentially be unaltered. An additional caveat that may prove to significantly hamper interpretation of the outcomes of the current ongoing clinical trials is that chloroquine/hydroxychloroquine my fail to actually achieve levels in the tumor cell sufficient to inhibit autophagy. Finally, it is formally possible that sensitization to therapy that may be observed in select clinical trials could be occurring through off-target effects of the chloroquine or hydroxychloroquine and be unrelated to autophagy inhibition (18, 19). If chloroquine/hydroxychloroquine mediated sensitization does not actually reflect a direct consequence of autophagy inhibition, then the outcome of this type of clinical trial would not be interpretable within the underlying framework of autophagy modulation for therapeutic benefit.

An additional complicating factor is that there is currently no uniformly accepted methodology for monitoring autophagy inhibition in patients' tumors. Assessment of autophagy in cell culture is facilitated by multiple assays, including but not limited to transmission electron microscopy, LC3 fluorescence microscopy, tandem mRFP/mCherry GFP fluorescence microscopy, immunohistochemistry and SQSTM1/p62 and LC3 binding protein turnover assays (20). These approaches allow for evaluation of both autophagy induction and inhibition, the latter being critical where the cytoprotective actions of autophagy are the focus of the studies. The capacity to evaluate the onset of autophagy (and presumably its inhibition as well) in vivo utilizes a number of approaches that are similar to those for cell culture, specifically the analysis of GFP-LC3/Atg8 in transgenic mice systemically expressing GFP-LC3 or by transfection with GFP-LC3 plasmids, immunohistochemical detection of LC3 or other autophagic substrates such as SQSTM1/p62 in paraffin-embedded or fresh frozen tissue, standard immunoblotting of these substrates and analysis of autophagy in tissues ex vivo (20). Unfortunately, the clinical assessment of autophagy induction and/or inhibition does not appear to lend itself to any of the current methodologies.

There are at least three additional factors that are likely to complicate interpretation of the current clinical trials. One is the impact of autophagy induction and inhibition on the immune response, an issue that is far from being resolved. Studies from a number of investigators have suggested that factors secreted from autophagic cells, specifically damage associated molecular patterns (DAMPs) such as ATP and HMGB1 are critical for an effective immune response to eliminate the tumor (21, 22, 23, 24); consequently, autophagy *inhibition* could prove to be counterproductive in a patient with a functional immune system. In contrast, it has been reported that activation of autophagy in tumor cells may promote *escape* from immunosurveillance and consequently autophagy inhibition could facilitate a more effective response of the immune system in conjunction with direct sensitization to therapy (25).

Another factor to consider is the potential influence of autophagy in the tumor stroma on the response to therapy (26, 27). There appears to be evidence that autophagy in tumor stroma

promotes tumor growth by providing energy, suppressing the capacity of the tumor cell to undergo apoptosis as well as facilitating tumor invasiveness and metastatic potential (26, 27). If this is, in fact, the case, then interference with stromal cell autophagy should collaterally repress tumorigenicity.

Finally, autophagy has been shown to be beneficial to normal tissue function in terms of the removal of dysfunctional proteins and overall maintenance of cellular homeostasis (28). In fact, autophagic dysfunction has been associated with various neurodegenerative disorders such as Parkinson's Disease, lysosomal storage disorders, and possibly diabetes (28) as well as hepatic diseases such as viral hepatitis and hepatoceullar carcinoma (29). Since it is highly unlikely that pharmacological autophagy inhibitors would be tumor-selective in action, undesirable and possibly life threatening side effects could accompany a prolonged and sustained suppression of autophagy. Alternatively, if the autophagy inhibition is only transient, as may be the case using pharmacological agents solely during the course of therapy, the impact on normal tissue function may not prove to be a major clinical concern.

The outcome of clinical trials performed without consideration of the possibility that autophagy induced in patient tumors may *not* be cytoprotective (or that the agents being tested do not, in fact, modulate autophagy in the tumor cell in the clinical setting) raises the concern that negative outcomes could undermine efforts to consider this strategy within the framework of conventional (or targeted) therapies. Given that is currently unrealistic to attempt to stratify patients according to whether autophagy induced in a clinical malignancy is cytoprotective or cytotoxic (even assuming that all of the tested therapies actually are promoting autophagy in patient malignancies), we risk the possibility of overlooking the subsets of patients whose tumors may be susceptible to sensitization via autophagy inhibition. However, not only does this field still lack biomarkers to determine, pre-therapy, which patients might be responsive to autophagy inhibition, we are also essentially blind as to how to evaluate the biochemical or molecular characteristics of tumors that are responsive, limiting the potential utility of these clinical trials to further inform the targeted development of this therapeutic strategy.

Nonprotective Autophagy, an additional wrinkle in the ether

Previous work by our laboratory as well as that from the Thorburn research group has identified an additional functional form of autophagy, which we have termed "nonprotective" (4, 18, 30, 31). Unlike cytoprotective autophagy, where inhibition results in an enhanced response to the therapeutic agent, or cytotoxic autophagy where inhibition is anticipated to lessen the impact of the therapeutic agent, inhibition of nonprotective autophagy fails to influence drug or radiation sensitivity. This was found to be the case for cisplatin and radiation in 4T1 and Hs578t breast tumor cells, doxorubicin in MCF-7 breast tumor cells and radiation in a variety of tumor cells lacking functional p53 (18, 30, 31, 32).

A recent paper by Eng et al. (19) essentially confirms these findings identifying the nonprotective function of autophagy and further builds upon this concept in experiments where the ATG7 autophagy gene has been silenced in the A549 non-small cell lung cancer cell line. In studies where ionizing radiation as well as more than 30 drugs exhibiting a

variety of mechanisms were tested, it was found that (with only a few exceptions) inhibition of autophagy left the IC_{50} virtually unchanged. Although it is unclear whether all of the therapeutic modalities examined actually promoted autophagy in the A549 cells, it can be assumed that this would be the case for the vast majority of the tested drugs. Furthermore, although the influence of autophagy inhibition on drug activity was not tested in tumor bearing animals, the conclusions of this work are strengthened by the fact that ATG7 silencing clearly eliminated survival of the A549 cells under conditions of nutrient starvation.

While the outcome of these studies clearly support previous findings that identified the *nonprotective* function of autophagy (18, 30, 31, 32), studies by other laboratories, including our own, frequently identified cytoprotective autophagy in non-small cell lung cancer cell lines. For instance, we reported that radiation promoted protective autophagy in H460 non-small cell lung cancer cells (33). However, as we do observe that etoposide promotes the nonprotective form of autophagy in the same cell line (unpublished observations), our work tends to suggest that whether autophagy is protective or nonprotective in a particular tumor cell line may depend on the nature of the treatment modality. In other studies involving radiation-induced autophagy, where p53 was either induced or silenced in isogenic experimental systems, we reported that cytoprotective autophagy required the cells to express functional p53 whereas in cells that are either null or mutant in p53, radiation-induced autophagy was nonprotective (31).

In a 2011 study by Han at el (7) where gefitnib and erlotinib were shown to promote autophagy in A549 and H1299 non-small cell lung cancer cell lines, chloroquine as well as silencing of ATG5 and ATG7 enhanced sensitivity to these tyrosine kinase inhibitors. These findings were confirmed and extended in a report by Zou et al (8) where sensitivity to erlotinib was increased by silencing of ATG5 in H460 and A549 cells and by exposure to chloroquine in H460, A549, H358 and H322 cells. In the latter work, the presence of chloroquine also substantially increased apoptosis; however, the effect of ATG5 silencing on apoptosis was minimal, involving no more than 10% of the cell population. Both Ren et al (9) and Wu et al (10) reported that 3-methyl adenine enhanced sensitivity to cisplatin in A549 cells, albeit in an A549 cell line that had been selected for cisplatin resistance; however, there were no confirmatory genetic silencing studies and it is recognized that 3-MA is not necessarily specific for autophagy as a cellular target. Wang et al (11) showed modest sensitization to topotecan and enhancement of apoptosis in A549 cells by chloroquine and with genetic silencing of ATG5. Pan et al (12) reported sensitization to 5-fluorouracil by 3-MA and by silencing of ATG7 in A549 cells along with an increase in apoptotic cell death while Park et al (13) reported sensitization to pemetrexed, also by 3-MA, in A549 cells. The divergence in experimental outcomes suggests that it cannot be assumed that the recent findings reported by Eng et al in A549 cells (19) can be extrapolated to conclude that autophagy in non-small cell lung cancer is uniformly nonprotective. Nonetheless, taken together, these reports clearly indicate that autophagy in response to radiation or chemotherapy can be either cytoprotective or nonprotective in function.

There is a quite extensive body of literature where the impact of autophagy has been evaluated using a spectrum of chemotherapeutic drugs or radiation in various experimental

tumor models, primarily cell culture and tumor xenografts. Suffice it to say, when examined carefully, the results of these studies are, at the very least, inconsistent in terms of whether autophagy induced by chemotherapy and radiation is cytoprotective and amenable to manipulation for therapeutic benefit. While it is beyond the scope of this commentary to summarize the outcome of these studies, we have recently published a focused review of the literature relating to autophagy inhibition in non-small cell lung cancer models in response to chemotherapy and radiation both in cell culture and in vivo (34). The reader is also directed to a recently published review relating to the capacity of autophagy inhibition to sensitize tumors to radiation (35)

Where do we go from here?

It is understandable that the oncology community has hastened to initiate clinical trials of autophagy inhibition as an adjunct to standard therapies, working under the premise that autophagy could represent a global mechanism of drug and radiation resistance. However, in retrospect, given the existence of at least three functional forms of autophagy in response to chemotherapy and radiation (cytoprotective, cytotoxic and nonprotective), the issue now is clearly considerably more complex. Fundamentally, the challenge is how to determine when therapy-induced autophagy is actually cytoprotective in patients in order to identify those patients for whom autophagy inhibition might prove to be beneficial. The governmental website (Clinical trials.org) indicates that clinical trials involving autophagy inhibition are ongoing involving, for example gemcitabine/Abraxane in pancreatic cancer, chemoradiation for glioblastoma, RAD001 in renal cell carcinoma, Ixabepilone in metastatic breast cancer, and FOLFOX/Bevacizumab in colorectal cancer. We have recently commented on the results of published clinical trials relating to autophagy inhibition, where we noted that “it might be prudent to develop a consensus based on preclinical data as to which types of cancer and which class or classes of drugs used in standard regimens might be most appropriate for testing in the context of clinical trials of HCQ or other modulators of autophagy” (17).

The strategy of autophagy inhibition would greatly benefit from the identification of serum markers that might be indicative of cytoprotective autophagy in response to a first round of therapy. However, we are currently far removed from knowing what form such markers might take since even established disease markers such as prostate specific antigen for prostate cancer have somewhat controversial prognostic significance. This is not to say that this goal cannot be achieved as it is now feasible to predict which patients might be susceptible to trastuzumab based on Her/neu expression in breast cancer and to tyrosine kinase inhibitors such as Gefitinib and erlotinib in lung cancer based on EGFR status. However, even in these more mature areas of oncology, a determination of the appropriate therapy is dependent on a tumor biopsy. In the case of the different forms of autophagy, the appropriate marker or panel of markers remains to be identified.

Given the limitations of our current knowledge in this field, the most effective strategy might be to obtain a biopsy with a sufficient number of cells that could be grown in culture and tested for responsiveness to a particular drug or panel of drugs in the absence and presence of an autophagy inhibitor. Not only would this provide information as to the nature of the autophagy induced by the therapy, but presumably this approach would also indicate

whether the autophagy inhibitor is effective in sensitizing the tumors to the therapy. Furthermore, an indication as to the extent of sensitization that might be anticipated is likely to provide guidance for deciding whether there is likely to be therapeutic value in proceeding with this approach.

It is, of course, possible that some classes of malignancies are generally susceptible to autophagy inhibition as a sensitization strategy, although preclinical data in cell culture suggest this is unlikely to be the case. How this would be determined for patients is unclear. The current strategy adopted by the NIH as well as many laboratories involves the testing of therapeutic strategies utilizing patient derived tumors grown as xenografts (PDXs), which is thought to be more predictive of clinical outcomes than most previous models (36, 37). In this context, in a study by Zinn et al (38) chloroquine was shown to sensitize a small cell lung cancer xenograft model to the Bcl-2 inhibitor ABT-737, but not when using patient derived tumor xenografts.

One strategy might be to screen large samples of patient derived tumors from a particular malignancy with a panel of drugs that are generally used for that disease to determine whether autophagy is induced and/or whether an autophagy inhibitor enhances drug or radiation sensitivity. However, even when these tumors are studied in tumor bearing animals, the potential involvement of the immune system will not be factored into the outcome since xenografts are obligatorily grown in immune suppressed models; nevertheless, as indicated above, it is possible if not likely that the immune response will be the ultimate determinant as to whether the strategy of autophagy inhibition will be successful in improving patient response to chemotherapy or radiation. It further remains a matter of conjecture as to what percentage of patient derived tumors would have to show positive responses and the extent of these responses that might be necessary for autophagy inhibition to be considered as having clinical applicability.

Given the relative paucity of preclinical information as to what might constitute cytoprotective autophagy in a patient’s malignancy (versus the cytotoxic and nonprotective forms), even if subpopulations of patients have disease that responds positively to autophagy inhibition in combination with conventional therapies, we are currently unable to identify what genetic background of the tumor or biochemical/molecular characteristics could be utilized as predictive factors for further application of this therapeutic strategy. This type of information (along with an assessment of the immune response and attention to the impact of autophagy on normal tissue), will ultimately be critical to providing the framework necessary to interpret whether the ongoing clinical trials will ultimately support the incorporation of pharmacological autophagy inhibitors as a component of cancer therapy.

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REFERENCES

1. Anding AL, Baehrecke EH. Autophagy in cell life and cell death. Curr Top Dev Biol. 2015; 114:67–91. Review. [PubMed: 26431564]
2. Ozpolat B, Benbrook DM. Targeting autophagy in cancer management - strategies and developments. Cancer Manag Res. 2015; 7:291–299. Review. [PubMed: 26392787]
3. Gewirtz DA. The autophagic response to radiation: relevance for radiation sensitization in cancer therapy. Radiat Res. 2014; 182:363–367. [PubMed: 25184372]
4. Gewirtz DA. The four faces of autophagy: implications for cancer therapy. Cancer Res. 2014; 74:647–651. [PubMed: 24459182]
5. Sharma K, Le N, Alotaibi M, Gewirtz DA, et al. Cytotoxic autophagy in cancer therapy. Int J Mol Sci. 2014; 15:10034–10051. [PubMed: 24905404]
6. Bae H, Guan JL. Suppression of autophagy by FIP200 deletion impairs DNA damage repair and increases cell death upon treatments with anticancer agents. Mol Cancer Res. 2011; 9:1232–1241. [PubMed: 21807966]
7. Han W, Pan H, Chen Y, Sun J, Wang Y, Li J, et al. EGFR tyrosine kinase inhibitors activate autophagy as a cytoprotective response in human lung cancer cells. PLoS One. 2011; 6:e18691. [PubMed: 21655094]
8. Zou Y, Ling YH, Sironi J, Schwartz EL, Perez-Soler R, Piperdi B. The autophagy inhibitor chloroquine overcomes the innate resistance of wild-type EGFR non-small-cell lung cancer cells to erlotinib. J Thorac Oncol. 2013; 8:693–702. [PubMed: 23575415]
9. Ren JH, He WS, Nong L, Zhu QY, Hu K, Zhang RG, et al. Acquired cisplatin resistance in human lung adenocarcinoma cells is associated with enhanced autophagy. Cancer Biother Radiopharm. 2010; 25:75–80. [PubMed: 20187799]
10. Wu T, Wang MC, Jing L, Liu ZY, Guo H, Liu Y, et al. Autophagy facilitates lung adenocarcinoma resistance to cisplatin treatment by activation of AMPK/mTOR signaling pathway. Drug Des Devel Ther. 2015; 9:6421–6431.
11. Wang Y, Peng RQ, Li DD, Ding Y, Wu XQ, Zeng YX, et al. Chloroquine enhances the cytotoxicity of topotecan by inhibiting autophagy in lung cancer cells. Chin J Cancer. 2011; 30:690–700. [PubMed: 21959046]
12. Pan X, Zhang X, Sun H, Zhang J, Yan M, Zhang H. Autophagy inhibition promotes 5-fluorouracil-induced apoptosis by stimulating ROS formation in human non-small cell lung cancer A549 cells. PLoS One. 2013; 8:e56679. [PubMed: 23441212]
13. Park JH, Lee YR, So HS, Lee KK, Lee SY, Moon SR, Jo HJ, Lee S, Jeong K, Kwon KB, Yang SH. The role of autophagy induced by pemetrexed in lung adenocarcinoma cells. Oncol Rep. 2014; 31:2365–2370. [PubMed: 24626722]
14. Thorburn A. Apoptosis and autophagy: regulatory connections between two supposedly different processes. Apoptosis. 2008; 13:1–9. Review. [PubMed: 17990121]
15. Jain MV, Paczulla AM, Klonisch T, et al. Interconnections between apoptotic, autophagic and necrotic pathways: implications for cancer therapy development. J Cell Mol Med. 2013; 17:12–29. [PubMed: 23301705]
16. Gewirtz DA. When cytoprotective autophagy isn't…and even when it is. Autophagy. 2014; 10:391–392. [PubMed: 24419177]
17. Poklepovic A, Gewirtz DA. Outcome of early clinical trials of the combination of hydroxychloroquine with chemotherapy in cancer. Autophagy. 2014; 10:1478–1480. [PubMed: 24991829]
18. Maycotte P, Aryal S, Cummings CT, Thorburn J, Morgan MJ, Thorburn A. Chloroquine sensitizes breast cancer cells to chemotherapy independent of autophagy. Autophagy. 2012; 8:200–212. [PubMed: 22252008]
19. Eng CH, Wang Z, Tkach D, Toral-Barza L, Ugwonali S, Liu S, et al. Macroautophagy is dispensable for growth of KRAS mutant tumors and chloroquine efficacy. Proc Natl Acad Sci U S A. 2016; 113:182–187. [PubMed: 26677873]

20. Klionsky DJ, Abdelmohsen K, Abe A, Abedin MJ, Abeliovich H, Acevedo Arozena A, et al. Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). Autophagy. 2016; 12:1–222. [PubMed: 26799652]
21. Hou W, Zhang Q, Yan Z, Chen R, Zeh IHJ, Kang R, et al. Strange attractors: DAMPs and autophagy link tumor cell death and immunity. Cell Death Dis. 2013; 4:e966. Review. [PubMed: 24336086]
22. Fucikova J, Moserova I, Urbanova L, Bezu L, Kepp O, Cremer I. Prognostic and Predictive Value of DAMPs and DAMP-Associated Processes in Cancer. Front Immunol. 2015; 6:402. Review. [PubMed: 26300886]
23. Michaud M, Xie X, Bravo-San Pedro JM, Zitvogel L, White E, Kroemer G. An autophagy-dependent anticancer immune response determines the efficacy of melanoma chemotherapy. Oncoimmunology. 2014; 3:e944047. [PubMed: 25610726]
24. Ko A, Kanehisa A, Martins I, Senovilla L, Chargari C, Dugue D, et al. Autophagy inhibition radiosensitizes in vitro, yet reduces radioresponses in vivo due to deficient immunogenic signaling. Cell Death Differ. 2014; 21:92–99. [PubMed: 24037090]
25. Tittarelli A, Janji B, Van Moer K, Noman MZ, Chouaib S. The Selective Degradation of Synaptic Connexin 43 Protein by Hypoxia-induced Autophagy Impairs Natural Killer Cell-mediated Tumor Cell Killing. J Biol Chem. 2015; 290:23670–23679. [PubMed: 26221040]
26. Zhao X1, He Y, Chen H. Autophagic tumor stroma: mechanisms and roles in tumor growth and progression. Int J Cancer. 2013; 132:1–8. [PubMed: 22684793]
27. Maes H, Rubio N, Garg AD, Agostinis P. Autophagy: shaping the tumor microenvironment and therapeutic response. Trends Mol Med. 2013; 19:428–446. [PubMed: 23714574]
28. Esteves, RA.; Oliveira, CR.; Cardoso, SM. Autophagy, the “Master” Regulator of Cellular Quality Control: What Happens when Autophagy Fails?. In: Bailly, editor. Autophagy - A Double-Edged Sword - Cell Survival or Death?. InTech;
29. Czaja MJ, Ding WX, Donohue TM Jr, Friedman SL, Kim JS, Komatsu M. Functions of autophagy in normal and diseased liver. Autophagy. 2013; 9:1131–1158. [PubMed: 23774882]
30. Bristol ML, Emery SM, Maycotte P, Thorburn A, Chakradeo S, Gewirtz DA. Autophagy inhibition for chemosensitization and radiosensitization in cancer: do the preclinical data support this therapeutic strategy? J Pharmacol Exp Ther. 2013; 344:544–552. [PubMed: 23291713]
31. Chakradeo S, Sharma K, Alhaddad A, Bakhshwin D, Le N, Harada H, et al. Yet another function of p53: The switch that determines whether radiation-induced autophagy will be cytoprotective or nonprotective. Implications for autophagy inhibition as a therapeutic strategy. Mol Pharmacol. 2015; 87:803–814. [PubMed: 25667224]
32. Maycotte P, Gearheart CM, Barnard R, Aryal S, Mulcahy Levy JM, Fosmire SP, et al. STAT3-mediated autophagy dependence identifies subtypes of breast cancer where autophagy inhibition can be efficacious. Cancer Res. 2014; 74:2579–2590. [PubMed: 24590058]
33. Sharma K, Goehe RW, Di X, Hicks MA 2nd, Torti SV, Torti FM, et al. 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. Autophagy. 2014; 10:2346–2361. [PubMed: 25629933]
34. Saleh T, Cuttino L, Gewirtz DA. Autophagy is not uniformly cytoprotective: a personalized medicine approach for autophagy inhibition as a therapeutic strategy in non-small cell lung cancer. Biochim Biophys Acta. 2016 Jun 14. [Epub ahead of print] Review.
35. Ondrej M, Cechakova L, Durisova K, Pejchal J, Tichy A. To live or let die: Unclear task of autophagy in the radiosensitization battle. Radiother Oncol. 2016; 119:265–275. [PubMed: 26993419]
36. Bousquet G, Janin A. Patient-Derived Xenograft: An Adjuvant Technology for the Treatment of Metastatic Disease. Pathobiology. 2016; 83:170–176. [PubMed: 27010922]
37. Cho SY, Kang W, Han JY, Min S, Kang J, Lee A, Kwon JY, Lee C, Park H. An integrative approach to precision cancer medicine using patient-derived xenografts. Mol Cells. 2016; 39:77–86. Review. [PubMed: 26831452]
38. Zinn RL, Gardner EE, Dobromilskaya I, Murphy S, Marchionni L, Hann CL, Rudin CM. Combination treatment with ABT-737 and chloroquine in preclinical models of small cell lung cancer. Mol Cancer. 2013; 12:16. [PubMed: 23452820]