

Autophagy: When to strike?

Shadi Zahedi^{1,2}, Jean M. Mulcahy Levy^{1,2,3*}

¹Department of Pediatrics, University of Colorado School of Medicine, Aurora, CO 80045, USA

²Morgan Adams Foundation Pediatric Brain Tumor Research Program, Children's Hospital Colorado, Aurora, USA

³Department of Pharmacology, University of Colorado School of Medicine, Aurora, CO 80045, USA

*Correspondence should be addressed to Jean M. Mulcahy Levy; Jean.MulcahyLevy@cuanschutz.edu

Received date: January 11, 2020, **Accepted date:** February 06, 2020

Copyright: © 2020 Zahedi S, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Autophagy was originally viewed as a widely conserved multistep lysosomal degradation pathway in eukaryotes. It includes the formation of autophagosomes, double-membrane structures engulfing cytoplasm with damaged organelles during the degradation process. Under normal conditions, autophagy is a mechanism of cell survival, however, the role of autophagy in human disease remains complicated.

In cancer, autophagy seems to have a dual role in tumor cell survival and death. During early stages of tumorigenesis, autophagy can limit tumor growth, however, in advanced cancers it may facilitate tumor progression as a protective mechanism against various stress conditions [1]. Given that tumors are frequently exposed to environmental stresses such as nutrient deprivation, low PH and hypoxic conditions, inhibiting autophagy appears to be a promising target for therapy. In fact, we and others have shown that targeting this pathway in combination with existing therapies can improve therapeutic outcome in some cancers [2-6].

In addition, there are somatic mutations that would predispose sensitivity to autophagy inhibition in certain tumor types. We have previously shown that BRAF^{V600E} makes pediatric central nervous system (CNS) tumor cells sensitive to autophagy inhibition as they demonstrate high rates of autophagy compared to wild-type cells [2]. We also have demonstrated *in vitro*, *ex vivo*, and in patients that autophagy inhibition overcomes multiple molecularly distinct resistance mechanisms to BRAF inhibition in BRAF mutant CNS tumors. Particularly, there was a synergistic effect between BRAFi and autophagy inhibition [4]. Other groups have also shown

the importance of autophagy in RAS mutant cancers as a key resistance mechanism to MEK or ERK inhibition. Combined autophagy inhibition in addition to ERKi and MEKi resulted in potent cytotoxicity in those models [5,6]. Current research efforts have mostly focused on utilizing chloroquine (CQ) or its derivatives such as hydroxychloroquine (HCQ) to inhibit late stage autophagy. However, lack of specificity, dose limiting cytotoxicity in combination with cytotoxic chemotherapy and inconsistency in autophagy inhibition across tumor types continues to be a challenge for the clinical use of these drugs [1]. Further studies have demonstrated differential effects of early versus late stage autophagy inhibition on tumor cell killing [7].

Together, these studies demonstrate how it is more essential to determine if inhibiting earlier phases of autophagy (involved in autophagosome formation) or later phases (involved in autophagosome cargo digestion) would yield better therapeutic outcomes. In our research studies, we aim to determine the optimal point to target and disrupt autophagy in BRAF^{V600E} brain tumor cells in order to improve patient outcomes. Our most recent data were able to demonstrate the effectiveness of early stage autophagy inhibition against ULK1 and VPS34, two early autophagy regulators, using SBI-0206965 and VPS34-IN1 respectively [3]. Both genetic and pharmacologic inhibition of early stage autophagy, particularly in the presence of BRAFi, reduced tumor cell growth and enhanced tumor cell death in BRAF mutant CNS tumor cells irrespective of their RAFi sensitivity. Interestingly, we observed increased treatment efficacy using early stage autophagy inhibitors in cells under stress (nutrient deprivation) which mirrors the tumor

microenvironment. Considering that others have shown a synergistic effect between ULK1 inhibition and mTOR inhibition [8,9], additional studies will be important to determine if we could increase treatment efficacy using mTOR inhibitors in combination with these early stage autophagy inhibitors in CNS tumors. These data suggest early stage autophagy inhibition may be a viable target in autophagy dependent CNS tumors.

As more specific and optimized autophagy inhibitors are being developed, future studies will directly compare early and late stage autophagy inhibition to determine optimal targets in autophagy dependent BRAF mutant CNS tumors. Considering development of resistance to standard therapies remains a challenge even in combination targeted therapies, the need for developing the most effective combination therapies gains considerable importance. In combination with autophagy inhibition, studies to investigate targeting additional pathways such as those involved in other stress responses and even harnessing the immune response to improve treatment outcomes are important.

Initially, both cytotoxic innate and adaptive immune systems can control tumor development. Tumor-associated danger signals result in acute inflammatory responses leading to tumor cell recognition, cytokine secretion (specifically, interleukin-12 (IL-12) and interferon- γ (IFN- γ), and tumor cell killing by natural killer (NK) cells, dendritic cells (DCs), and macrophages. After migrating to nearby lymph nodes, Mature DCs present tumor antigens and activate CD4⁺ and CD8⁺ T cells which will then migrate to tumor site and facilitate tumor cell killing [10].

Some tumor cells may manage to evade immune system attacks through developing various mechanisms and replicate leading to clinically detectable tumors [11]. In addition to the contribution of hypoxic and immunosuppressive microenvironment, cancer cells may down-regulate tumor associated antigens (TAAs) and major histocompatibility complex (MHC) class I expression leading to the acquaintance of low immunogenicity [12]. Additionally, tumor cells may develop resistance by suppressing CD4⁺ and CD8⁺ T cells via immunosuppressive cytokines (such as IL-10), factors regulating lymphocyte chemotaxis or immune check points such as programmed cell death protein 1 (PD1) facilitating the differentiation of immunosuppressive regulatory T cells [13].

It has been reported that autophagy can regulate immune system components, in particular NK cells, DCs, and T and B lymphocytes. By influencing their survival, activation, proliferation, differentiation, and homeostasis, autophagy

can affect innate and adaptive immune responses. For example, initiation of tumor growth has been associated with decreased autophagy and infiltration of regulatory T cells that suppress the immune system [14]. It can also impact the release of cytokines and antibodies. Cytokines can also stimulate the early stages of autophagy but block autophagy flux (or the completion of the cycle) which in turn aggravates ER stress and increases lysosomal cell death [15]. It is important to note that a number of cytokines, immunoglobulins, and immune-related cells in turn affect the function of autophagy. For instance, transforming growth factor (TGF)- β , IFN- γ , IL-1, IL-2, and IL-12 are considered as autophagy inducers and IL-4, IL-10, and IL-13 can act as autophagy inhibitors [16].

The exact role or interaction between autophagy and the body's immune response to tumors remains in debate. On one side, it's possible that effective autophagy is needed to stimulate tumor recognition by the immune system [17,18]. It has also been shown that autophagy supports antigen presentation and a potential improved immune response [19]. Inhibition of autophagy could, in theory, blunt these responses. In contrast, it has been shown that autophagy inhibition during immunotherapy can enhance sustained tumor regression [20]. Targeted autophagy inhibition in T-cells can enhance an anti-tumor immune response by increasing the shift to effector memory cells and increasing production of interferon- γ [21]. Studies utilizing both early and late stage autophagy inhibitors have demonstrated immune reactivation against tumors. For instance, a recent report showed that lysosomes limited anticancer efficacy of CD8⁺ T cells in melanoma. Also, in melanoma, upregulation of autophagy by hypoxia resulted in diminished cell death induced by immune effectors. Treatment with HCQ enhanced tumor cell killing under this hypoxic condition [22]. Studies have shown that beclin1, a key component of early stage autophagy, results in an increase in T cell infiltration into the tumor microenvironment [23]. Finally, there are studies that find an equivalent T-cell response with and without autophagy inhibition [24].

Even though immunotherapeutic strategies aimed at boosting anti-tumor immunity are promising, immune tolerance remains a major challenge in cancer immunotherapy. As immunologic tolerance molecules such as IDO, CTLA-4, and PD-1 can regulate immune tolerance through autophagy pathways, it is key to understand the relationship between autophagy and tumor immune tolerance to design the most effective treatment strategy [15]. For instance, PD-1, a T-cell inhibitory checkpoint molecule, interacts with PDL-1 on the surface of the tumor cells suppressing an anti-tumor response. Recent studies have shown that blocking PD-1/

PDL-1 axis via anti-PD-1 and anti-PDL-1 antibodies triggers autophagy in tumor cells and the addition of autophagy inhibitors can serve as an attractive combination immunotherapy approach [25]. Other studies have demonstrated anti-PDL-1 as a potential biomarker for response to mTOR or autophagy inhibitors in selected cancers [25].

Although, emerging evidence from cancer immunotherapy clinical trials has highlighted the crucial role of T cells in tumor elimination, most encouraging results have been in the context of hematological cancers and more recently in melanoma. Improving responses in CNS tumors continues to be complex with additional issues such as how to traffic the appropriate immune cells from the periphery into the brain [26]. And once the correct cells are in the CNS, how do we make them work better? There is a clear, although complex, connection between autophagy and the tumor immune response. We have clearly shown that both early and late stage autophagy inhibition are effective in autophagy dependent CNS tumors, such as those with BRAF mutations [2,3]. But can these responses be improved with a better understanding of the link between these pathways and the immune system? Early studies in melanoma have already investigated triple therapy with BRAF, MEK and PD-1 blockade and shown improved tumor control [27]. Is it possible to further these responses with autophagy manipulation? Future studies are ongoing to answer these questions and it will be important to include the analysis of anti-tumor immune responses in ongoing and future clinical trials where we are manipulating autophagy.

References

1. Levy JM, Thorburn A. Autophagy in cancer: moving from understanding mechanism to improving therapy responses in patients. *Cell Death & Differentiation.* 2019 Dec 13;1-5.
2. Levy JM, Thompson JC, Griesinger AM, Amani V, Donson AM, Birks DK, Morgan MJ, Mirsky DM, Handler MH, Foreman NK, Thorburn A. Autophagy inhibition improves chemosensitivity in BRAFV600E brain tumors. *Cancer Discovery.* 2014 Jul 1;4(7):773-80.
3. Zahedi S, Fitzwalter BE, Morin A, Grob S, Desmarais M, Nellan A, Green AL, Vibhakar R, Hankinson TC, Foreman NK, Levy JM. Effect of early-stage autophagy inhibition in BRAF V600E autophagy-dependent brain tumor cells. *Cell Death & Disease.* 2019 Sep 12;10(9):1-5.
4. Levy JM, Zahedi S, Griesinger AM, Morin A, Davies KD, Aisner DL, Kleinschmidt-DeMasters BK, Fitzwalter BE, Goodall ML, Thorburn J, Amani V. Autophagy inhibition overcomes multiple mechanisms of resistance to BRAF inhibition in brain tumors. *Elife.* 2017 Jan 17;6:e19671.
5. Wang Y, Gallant RC, Ni H. Extracellular matrix proteins in the regulation of thrombus formation. *Current opinion in hematology.* 2016 May 1;23(3):280-7.
6. Kinsey CG, Camolotto SA, Boespflug AM, Guillen KP, Foth M, Truong A, Schuman SS, Shea JE, Seipp MT, Yap JT, Burrell LD. Protective autophagy elicited by RAF → MEK → ERK inhibition suggests a treatment strategy for RAS-driven cancers. *Nature Medicine.* 2019 Apr;25(4):620-7.
7. Goodall ML, Fitzwalter BE, Zahedi S, Wu M, Rodriguez D, Mulcahy-Levy JM, Green DR, Morgan M, Cramer SD, Thorburn A. The autophagy machinery controls cell death switching between apoptosis and necroptosis. *Developmental Cell.* 2016 May 23;37(4):337-49.
8. Egan DF, Chun MG, Vamos M, Zou H, Rong J, Miller CJ, Lou HJ, Raveendra-Panickar D, Yang CC, Sheffler DJ, Teriete P. Small molecule inhibition of the autophagy kinase ULK1 and identification of ULK1 substrates. *Molecular Cell.* 2015 Jul 16;59(2):285-97.
9. Martin KR, Celano SL, Solitro AR, Gunaydin H, Scott M, O'Hagan RC, Shumway SD, Fuller P, MacKeigan JP. A potent and selective ULK1 inhibitor suppresses autophagy and sensitizes cancer cells to nutrient stress. *Iscience.* 2018 Oct 26;8:74-84.
10. Wang M, Zhang C, Song Y, Wang Z, Wang Y, Luo F, Xu Y, Zhao Y, Wu Z, Xu Y. Mechanism of immune evasion in breast cancer. *OncoTargets and Therapy.* 2017;10:1561.
11. Gonzalez H, Hagerling C, Werb Z. Roles of the immune system in cancer: from tumor initiation to metastatic progression. *Genes & Development.* 2018 Oct 1;32(19-20):1267-84.
12. Sethumadhavan S, Silva M, Philbrook P, Nguyen T, Hatfield SM, Ohta A, Sitkovsky MV. Hypoxia and hypoxia-inducible factor (HIF) downregulate antigen-presenting MHC class I molecules limiting tumor cell recognition by T cells. *PLoS One.* 2017;12(11).
13. Rabinovich GA, Gabrilovich D, Sotomayor EM. Immunosuppressive strategies that are mediated by tumor cells. *Annual Review of Immunology.* 2007 Apr 23;25:267-96.
14. Rao S, Tortola L, Perlot T, Wirnsberger G, Novatchkova M, Nitsch R, Sykacek P, Frank L, Schramek D, Komnenovic V, Sigl V. A dual role for autophagy in a murine model of lung cancer. *Nature Communications.* 2014 Jan 20;5(1):1-5.

15. Jiang GM, Tan Y, Wang H, Peng L, Chen HT, Meng XJ, Li LL, Liu Y, Li WF, Shan H. The relationship between autophagy and the immune system and its applications for tumor immunotherapy. *Molecular Cancer.* 2019 Dec;18(1):17.
16. Harris J. Autophagy and cytokines. *Cytokine.* 2011 Nov 1;56(2):140-4.
17. Ko A, Kanehisa A, Martins I, Senovilla L, Chargari C, Dugue D, Marino G, Kepp O, Michaud M, Perfettini JL, Kroemer G. Autophagy inhibition radiosensitizes in vitro, yet reduces radioresponses in vivo due to deficient immunogenic signalling. *Cell Death & Differentiation.* 2014 Jan;21(1):92-9.
18. Michaud M, Martins I, Sukkurwala AQ, Adjemian S, Ma Y, Pellegatti P, Shen S, Kepp O, Scoazec M, Mignot G, Rello-Varona S. Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. *Science.* 2011 Dec 16;334(6062):1573-7.
19. Li Y, Hahn T, Garrison K, Cui ZH, Thorburn A, Thorburn J, Hu HM, Akporiaye ET. The vitamin E analogue α -TEA stimulates tumor autophagy and enhances antigen cross-presentation. *Cancer Research.* 2012 Jul 15;72(14):3535-45.
20. Liang X, De Vera ME, Buchser WJ, de Vivar Chavez AR, Loughran P, Stolz DB, Basse P, Wang T, Van Houten B, Zeh HJ, Lotze MT. Inhibiting systemic autophagy during interleukin 2 immunotherapy promotes long-term tumor regression. *Cancer Research.* 2012 Jun 1;72(11):2791-801.
21. DeVorkin L, Pavey N, Carleton G, Comber A, Ho C, Lim J, McNamara E, Huang H, Kim P, Zacharias LG, Mizushima N. Autophagy regulation of metabolism is required for CD8⁺ T cell anti-tumor immunity. *Cell Reports.* 2019 Apr 9;27(2):502-13.
22. Pan H, Chen L, Xu Y, Han W, Lou F, Fei W, Liu S, Jing Z, Sui X. Autophagy-associated immune responses and cancer immunotherapy. *Oncotarget.* 2016 Apr 19;7(16):21235.
23. Janji B, Berchem G, Chouaib S. Targeting autophagy in the tumor microenvironment: new challenges and opportunities for regulating tumor immunity. *Frontiers in Immunology.* 2018 Apr 25;9:887.
24. Starobinets H, Ye J, Broz M, Barry K, Goldsmith J, Marsh T, Rostker F, Krummel M, Debnath J. Antitumor adaptive immunity remains intact following inhibition of autophagy and antimalarial treatment. *The Journal of Clinical Investigation.* 2016 Dec 1;126(12):4417-29.
25. Robainas M, Otano R, Bueno S, Ait-Oudhia S. Understanding the role of PD-L1/PD1 pathway blockade and autophagy in cancer therapy. *OncoTargets and Therapy.* 2017;10:1803.
26. Ratnam NM, Gilbert MR, Giles AJ. Immunotherapy in CNS cancers: the role of immune cell trafficking. *Neuro-oncology.* 2019 Jan 1;21(1):37-46.
27. Ribas A, Lawrence D, Atkinson V, Agarwal S, Miller WH, Carlino MS, Fisher R, Long GV, Hodi FS, Tsoi J, Grasso CS. Combined BRAF and MEK inhibition with PD-1 blockade immunotherapy in BRAF-mutant melanoma. *Nature Medicine.* 2019 Jun;25(6):936-40.