Hypoxia-induced autophagy in tumor cells: a key target for improving cancer immunotherapy

Jerome Paggetti1, Elodie Viry1, Guy Berchem1,2, Etienne Moussay1, Bassam Janji1

1Laboratory of Experimental Hemato-Oncology, Department of Oncology, Public Research Center for Health, Luxembourg City, Luxembourg
2Centre Hospitalier de Luxembourg, Department of Hemato-Oncology, Luxembourg City, Luxembourg

Correspondence: Bassam Janji
E-mail: bassam.janji@crp-sante.lu
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Cancer was initially thought to be just a disease of cells with deregulated gene expression. It may be more accurate to consider also cancer as a disease of the microenvironment. It is now well documented that the tumor microenvironment plays a determinant role in cancer cell adaptation and resistance to therapies. In particular, hypoxia has been described to play a central role in activating multiple overlapping adaptive mechanisms leading to the emergence of resistant tumor cells able to outmaneuver an effective immune response and escape from immune cell killing. Despite the remarkable and fairly rapid progress over the past two decades regarding the role of the microenvironment in cancer biology and treatment, our understanding of its actual contribution to cancer resistance is still poor and fragmented. Moreover, the microenvironment is now considered to be of critical importance during the initiation and progression of carcinogenesis. By playing a key role in shaping and remodeling stroma reactivity and reprogramming phenotypic and functional plasticity, the tumor microenvironment represents therefore an important hallmark of cancer. A challenge for immunologists is to better understand tumor plasticity and immune-resistant tumor cell variants, which appear to be the greatest impediment to successful immunotherapy. In this context, autophagy has recently emerged as a new player in regulating the anti-tumor immune response in hostile tumor microenvironment. In this review, we summarize recent data describing how autophagy activation under hypoxia impairs the anti-tumor immune response. It is our belief that autophagy may represent a conceptual realm for new immunotherapeutic strategies aiming to block immune escape and therefore providing rational approach to future tumor immunotherapy design.

Keywords: Autophagy; Immune response; Tumor immunotherapy; Tumor microenvironment


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The resistance of cancer cells to anti-tumor immune responses in hostile tumor microenvironment implies that cancer cells activate several overlapping adaptation mechanisms to survive and/or neutralize immune-cell attack [1]. Recently, macroautophagy (hereafter referred to as autophagy) [2-4] has emerged as a new critical mechanism activated in tumor cells in hypoxic microenvironment that mediates tumor resistance to innate and adaptive anti-tumor immune responses. Autophagy acts as a catabolic process crucial for cellular homeostasis and maintenance of cell
Figure 1. Autophagy activation in tumor cell acts as an intrinsic resistance mechanism against anti-tumor immune response. The tumor microenvironment and/or EMT program activate autophagy in target cells. The induction of autophagy operates as a cell resistance mechanism leading to tumor escape from CTL- or NK-mediated lysis. A. Hypoxic stress leads to the accumulation of HIF-1α. HIF-1α activates autophagy and simultaneously increases the phosphorylation level of STAT3 at the Tyr705 residue. As an autophagic substrate, p62/SQSTM1 is degraded in the autophagosomes following their fusion with lysosomes. As p62 is involved in targeting pSTAT3 to the UPS, its degradation leads to the accumulation of pSTAT3 in cells and such accumulation constitutes a cell survival mechanism. In autophagy-defective cells, p62 is no longer degraded, and its accumulation accelerates the UPS-dependent degradation of pSTAT3 and thereby restores CTL-mediated tumor cell lysis. B. The acquisition of an EMT phenotype confers resistance to CTL-mediated lysis through autophagy induction. The increase in mesenchymal markers following the activation of EMT program leads to the up-regulation of BECN1 by a yet undefined mechanism. Such upregulation induces autophagy and impairs CTL-mediated tumor cell lysis. In mesenchymal cells, targeting BECN1 is sufficient to restore CTL-mediated lysis. C. Following the recognition of their targets, NK cells secrete cytotoxic granules containing PRF1, GZMB, and other hydrolytic enzymes that enter target cells, traffic to enlarged endosomes, and initiate tumor cell death. Under hypoxia, excessive autophagy in target cells leads to the fusion of autophagosomes with vesicles containing GZMB leading to its specific degradation by autophagy, thereby inhibiting NK-mediated lysis. Targeting autophagy prevents the degradation of GZMB and thereby restores NK-mediated tumor cell killing.

integrity under stressful conditions. Autophagy is a degradation mechanism of cell components which allows
the recycling of essential amino acids, nucleotides, and fatty acids necessary for energy and macromolecule biosynthesis [17,18]. During cancer progression, autophagy can be induced by different stresses, particularly hypoxia, nutrient deprivation, or extracellular matrix detachment [9,10]. The autophagic process is characterized by the formation of the phagophore or isolation membrane. Following this so-called nucleation stage, the phagophore is elongated by several Autophagy-related proteins (ATG) and the Microtubule-associated protein 1A/1B-light chain 3 (LC3)-I is lipidated into LC3-II. Maturation of the phagophore, through the action of LC3-II and BECN1 proteins, enables the sequestration of cell constituents into well-characterized vesicles named autophagosomes. Fusion of autophagosomes with lysosomes leads to the formation of autolysosomes and the degradation of their contents by lysosomal hydrolases [11].

Recent reports demonstrate that autophagy activation not only enables tumor cells to survive stress conditions during cancer development but also provides them an intrinsic resistance mechanism against anti-tumor immune response. First evidence for such a role of autophagy was provided by Noman et al. who demonstrated that hypoxic lung carcinoma cells can evade cytolytic T lymphocyte (CTL)-mediated lysis through autophagy induction [12,13]. Indeed, inhibition of autophagy using small interfering RNA (siRNA) directed against ATG5 or BECN1 restored tumor cells sensibility to CTL-mediated lysis which correlated with a decrease in hypoxia-dependent induction of the phosphorylation of Signal Transducer and Activator of Transcription (STAT)-3. This result allowed the prediction that blocking autophagy would inhibit pSTAT3-dependent survival mechanism making tumor cells more susceptible to CTL attack under hypoxia. However, considering the degradation role of autophagy, it is difficult to perceive that autophagy is involved in the stabilization of pSTAT3 under hypoxia. Focusing on the crosstalk between the adaptor protein p62/SQSTM1, the ubiquitin-proteasome system (UPS) and autophagy, this study revealed that the induction of hypoxia inducible factor (HIF)-1α has two effects in tumor cells: i) HIF-1α triggers the phosphorylation of Src which subsequently phosphorylates the tyrosine residue Y705 of STAT3; ii) HIF-1α activates autophagy by a mechanism implicating the increased expression of BCL2/adenovirus E1B 19 kDa protein-interacting protein (BNIP)3/BNIP3L and the dissociation of the BECN1-BCL2 (B cell lymphoma 2) complex. Autophagy activation results in degradation of the p62 protein. Knowing that p62 is the receptor/adaptor protein responsible for targeting pSTAT3 to the UPS, the autophagy-dependent degradation of p62 leads to the accumulation of pSTAT3. When autophagy is inhibited in tumor cells, the degradation of p62 is blocked and therefore accumulates in tumor cells. This accumulation accelerates the UPS-dependent degradation of pSTAT3 [14] (Figure 1A).

Epithelial to mesenchymal transition (EMT) is a transdifferentiation process necessary for the morphogenesis of tissue during embryonic development [15]. While its role in cancer cell invasion, metastasis and drug resistance is well established, recent report described that autophagy can be activated in tumor cells undergoing EMT and that such EMT-induced autophagy represents another mechanism of cancer cell resistance to CTL-mediated lysis [16,17]. In this study, the authors showed that the induction of EMT program by overexpression of SNAI1 in breast cancer cells coincides with a drastic change in cell morphology and the activation of autophagy flux most likely through the overexpression of BECN1 in mesenchymal cells. Although the exact molecular mechanism by which the EMT affects the expression of BECN1 remained to be addressed, several lines of evidence indicate that this may be related to SNAI1- or EMT-dependent repression of microRNA(s) involved in modulation of BECN1 expression [18,19]. This result extended the role of SNAI1 as a regulator of autophagy and paves the way to research related to the functional role of EMT-induced autophagy in tumor cells. In this context, results described in this study showed that targeting BECN1 in mesenchymal cells was sufficient to restore CTL-mediated tumor cell lysis, without affecting the mesenchymal morphology and the expression of EMT markers. This finding implies that autophagy is a downstream target of the EMT program in breast cancer cells. Overall, this study suggests that EMT-induced autophagy is a novel mechanism by which tumor cells regulate CTL reactivity and impede their cytotoxic activity, and further point to the complex relationship between the tumor and the immune system (Figure 1B).

It is now well established that several resistance mechanisms are regulated in tumor cells to escape immune surveillance in hypoxic tumor microenvironment. Recent evidence described how tumor cells can escape natural killer (NK)-mediated immune surveillance by activating autophagy under hypoxia [20,21]. Indeed, NK cells recognize and kill their targets by several mechanisms including the release of cytotoxic granules containing perforin (PRF1) and serine protease granzyme B (GZMB). It has been recently proposed that PRF1 and GZMB enter target cells by endocytosis and traffic to large endosomes named "gigantosomes" [22, 23]. Subsequently, PRF1 is involved in the formation of pores in the membrane of the "gigantosome", leading to the gradual release of GZMB and the initiation of apoptotic cell death. The formation of amphisomes following the fusion between autophagic vacuoles and early endosomes appears to be necessary in some cases for the generation of autolysosomes. In this report [20], the authors described that the pro-apoptotic protein GZMB is selectively degraded upon autophagy...
activation in hypoxic cells, thereby blocking NK-mediated target cell apoptosis (Figure 1C). In line with this, they showed that GZMB is detected in autophagosomes and provided evidence that GZMB level is significantly decreased in hypoxic compared to normoxic target cells. Furthermore, targeting autophagy or inhibiting lysosomal hydrolases by genetic or pharmacological approaches restored GZMB level which ultimately leads to the recovery of hypoxic cells lysis by NK cells in vitro and in vivo. Based on these results, the authors stated that tumor regression can be achieved by inhibiting autophagy in hypoxic cancer cells, thus enabling their NK-mediated lysis [20, 21].

Overall, studies described above underline the activation of autophagy as a key mechanism in tumor escape from immune cell attack within the tumor microenvironment. However, an important issue that arises from these studies is whether hypoxia is the only microenvironmental factor involved in the induction of autophagy in tumor cells. An interesting recent report provided strong evidence that lymphoid effectors not only provide lytic signals but also promote autophagy in the remaining target cells, a process called cell-mediated autophagy (C-MA) [24]. Thus, C-MA has been reported in different human epithelial tumors after interaction with immune cells at high ratio of effectors to targets. Importantly, it has been showed that C-MA not only acts as a mechanism of resistance to immune cell-mediated lysis but also limits the cytotoxic activity of stress factors such as γ-radiation [24].

These studies highlighted that the activation of autophagy plays a critical role in tumor cell escape from both adaptive and innate immunity. Therefore, targeting autophagy has been proposed to improve CTL- and NK-based immunotherapy in experimental mouse model [12, 13, 20]. Intense research efforts are currently focusing on the development of autophagy inhibitors that could improve tumor immunotherapy. Several pharmacological inhibitors, including chloroquine (CQ), hydroxychloroquine (HCQ) and quinacrine, have been described to improve therapeutic efficacy of several drugs in cancer patients [25, 26]. About fifty clinical trials are currently investigating the effect of autophagy inhibition in association with chemotherapy drugs (http://clinicaltrials.gov). For example, administration of high dose of interleukin-2 together with CQ is associated with an increase in long-term tumor regression [27].

In conclusion, activation of autophagy in cancer cells could represent a key target for improving efficacy of anti-tumor immunotherapies. Understanding the complete mechanism by which autophagy modulates tumor cell susceptibility to immune effectors could also generate new opportunities for therapeutic combinations.

**Conflicting interests**

The authors have declared that no competing interests exist.

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