Harnessing Autophagy for Melanoma Benefit

Marco Corazzari¹ and Penny E Lovat¹

Cutaneous melanoma, the most aggressive form of skin cancer, remains one of the most difficult human cancers to treat, with an increasing incidence in developed countries which has risen faster than any other malignancy over the past 40 years [1,2]. Melanoma occurs when the pigment-producing cells (melanocytes) within the basal epidermis become transformed due to both environmental and genetic risk factors [1,3] (Figure 1). Although early stage disease is treatable through surgical excision alone, metastatic melanoma is highly invasive and evolves with an extensive repertoire of molecular defences against immunological and cytotoxic attack [4], rendering this type of tumour notoriously unresponsive to conventional chemotherapy and leaving an acute need for novel therapeutic strategies [5,6,7].

Genetic alterations predisposing to melanocyte transformation and the onset of melanoma include those in PI3K/PTEN/AKT signalling pathways, and more frequently hyper activating mutations in NRAS/BRAF which result in constitutive activation of RAS/RAF/MAPK signalling. Indeed, 20% of all melanomas harbour activating NRAS mutations, while up to 70% harbour BRAF mutations (with BRAFV600E representing more than the 90% of these mutations) [6,7]. While NRAS and BRAF mutations are mutually exclusive, concomitant activation of BRAF and loss of PTEN leads to the spontaneous melanoma development, with the increased likelihood of both distal and systemic metastases [8]. However, the observation that mutant BRAFV600E is also detected in benign nevi, suggests that BRAF mutation alone is insufficient to induce tumorigenesis and that additional factors are required for cancer progression [9,10]. Moreover, despite the recent success of vemurafenib, a specific BRAFV600E inhibitor, chemoresistance evolves quickly [11] suggesting other compensatory signalling mechanisms contribute to tumour progression. Dysregulation of autophagy has accordingly been postulated as a secondary event contributing to melanoma progression [12-14] and, importantly, also plays a key role in chemoresistance [12,15-19].

Autophagy, represents the major lysosomal-mediated intracellular process for the bulk degradation and recycling of surplus proteins and aged or damaged organelles within the cytoplasm. Activated by nutrient deprivation this process is tightly regulated by an intricate cascade of events involving the mammalian target of rapamycin (mTOR), the ULK1 kinase complex and the Class III phosphatidylinositol 3-kinase (PI3K) complex, (including Beclin 1, ATG14, UV irradiation resistance-associated tumour suppressor gene (UVRAG), Ambra1 and the Bcl-2 family [20,21]) (which facilitate the formation of a double-membraned vesicle (autophagosome) around cytoplasmic material to be degraded [22-24] and its targeting for lysosomal degradation. Metabolic activity and cell survival are consequently sustained by the recycling of degradation products (Figure 2).

Though essential for the maintenance of cellular homeostasis under conditions of nutrient deprivation, paradoxically, autophagy promotes both tumour suppression and tumour development [25]. On one hand, autophagy represents an important barrier for cellular transformation, supported by data demonstrating mice deficient for Atg4C, Bif-1, TSC1/2, LKB1 and p53, or heterozygous for Beclin-1.
are tumour-prone. However, autophagy also sustains tumour growth contributing to the survival of tumour cells in a microenvironment which is commonly poor of oxygen and nutrients [4,25], typical in advanced stages of cancer.

Similar to other tumours, cutaneous melanomas rather than benign nevi display increased levels of autophagic activity as evidenced by studies showing increased levels of LC3B and the increased number of autophagosomes which are associated with the increased likelihood of metastasis and poor outcome [12,13,26]. In addition, several studies have shown down-regulation of cytoplasmic Beclin-1 parallels melanoma disease stage progression (Table 1) [27-29]. The BRAF status in melanoma also influences autophagy; mutant BRAFV600E increases basal levels of LC3-II but confers the resistance of metastatic melanoma cells to mTOR-dependent autophagy induction [19], the therapeutic significance of which is the relative inability of melanomas to further up-regulate autophagy following chemotherapy. In line with other tumours loss or deregulated autophagy thus promotes melanoma tumorigenesis while the regain of autophagic function in advanced stages of cancer sustains and promotes tumour growth and survival.

Current strategies to harness autophagy for clinical benefit have been primarily based on two observations, firstly that cancer cells use autophagy to survive metabolic stress and secondly that many chemotherapeutic drugs activate autophagy as a compensatory survival mechanism to counteract apoptotic signals (by removing damaged cellular structures and by reprogramming cellular energy metabolism to cope with therapeutic stress). Consequently autophagy inhibition may represent a viable therapeutic strategy for advanced stage melanomas. However, observations that metastatic melanoma cells with higher basal levels of autophagy are the less responsive to chemotherapy when the autophagic pathway is impaired coupled with the possible side effects of autophagy inhibition on secondary tumour development and the antitumor immune response, suggest autophagy induction may represent a more favourable approach [30-34]. To this end, pre clinical studies of Metformin, Terfenadine or THC treatment of melanoma, glioma or hepatocellular carcinoma offer valuable insight into how drug-induced autophagy can be harnessed to promote cancer cell death [31-34].

The optimal strategy of how to harness autophagy for clinical benefit thus likely depends on both tumour stage and type. Although the lysosomal inhibitor hydroxychloroquine represents a novel autophagy inhibitor, innovative compounds have been developed and used in clinical trials to specifically inhibit the process. Their preclinical evaluation alone and in combination with chemotherapeutic agents which induce pro-survival autophagy coupled with the awaited outcome of phase I/II trials of agents mediating cytotoxic autophagy will thus pave the way to the exciting development of novel and optimal therapeutic strategies through which to harness autophagy for the clinical benefit of melanoma.

### Table 1: Autophagy paradox in melanoma.

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References


**Author Affiliations**

1Department of Biology, University of Rome ‘Tor Vergata’, Rome, Italy
2National Institute for Infectious Diseases IRCCS “L. Spallanzani”, Rome, Italy
3Dermatological Sciences Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK