

Autophagy in Melanoma

James L Cox*

Department of Biochemistry, AT Still University, Kirksville, Missouri, USA

ABSTRACT

The incidence of melanoma is rising and it remains a very difficult cancer to treat effectively. There is a challenge in treating late-stage melanoma that concerns development of drug resistance to current therapies. Newer therapies will unlock therapeutic resistance by activating alternative cell death pathways in melanoma. Autophagy is a cell survival process for advanced melanoma. Hopefully, newer agents that are being experimentally tested will couple autophagic cell death with other anti-cancer therapies to produce synergistic killing effects for melanoma.

Keywords: Autophagy; Melanoma; Autophagosome; Apoptosis

DESCRIPTION

Autophagic flux can be estimated for melanoma cells via Western blot analysis of LC3 protein levels. In addition, autophagosome content of melanoma cells can be visualized by electron microscopy. A large study of melanoma tumors showed higher LC3 levels in metastases than in primary tumors [1-7]. Melanoma is particularly resistant to apoptosis inducing agents and therefore alternative death pathways are sought for more effective therapies. Several groups have looked at induction of autophagic (type II) cell death as a potential therapeutic avenue. There is still controversy whether true “death by autophagy” exists or excessive autophagy triggers apoptotic type cell death. CLIB-MECA, a selective A3 adenosine receptor agonist, with paclitaxel was able to induce not only apoptosis but also autophagic cell death in melanoma [8].

Autophagy-related protein 5 (ATG5) is often down-regulated in primary melanomas [9]. Expression of ATG5 correlates with prognosis and low ATG5 levels had reduced progression-free survival. High levels of ATG5 were found in aggressive melanomas both in tumor xenografts and tumor cell spheroids, but not in monolayer cultures [10]. Hydroxychloroquine (HCQ), an inhibitor of autophagy, treatment of aggressive cell tumor spheroids leads to death within the spheroids. Patients with melanomas that are more highly dependent on autophagy may have better outcomes when autophagy is inhibited in conjunction with other chemotherapeutics.

In human melanoma capsaicin not only induced apoptosis but also cytoprotective autophagy [11]. Inhibition of autophagy with 3-methyl adenine, 3-MA, increased caspase activation showing autophagy suppressed apoptosis. Perhaps one way forward is to use agents which both stimulate and inhibit autophagy to maximize killing effects. Autophagy is significantly increased in aggressive versus indolent melanomas. Blockade of autophagy with HCQ could dramatically increase cell death in conjunction with temozolomide treatment of aggressive melanoma cells in vitro [12]. In phase I clinical trials melanoma patients were treated with temozolomide and HCQ to both induce and block autophagy [13]. In some patients disease stabilization was found and the treatment was safe and tolerable. More trials of this nature need to be done with more potent autophagy inhibitors to see how vulnerable melanoma is to this type of therapy. This is necessary because HCQ has certain drawbacks and is difficult to maintain at effective levels.

Melanoma cells under acidic conditions increase autophagy to survive acid stress [14]. Knockdown of the autophagic ATG5 protein dramatically increased acid stress-induced cell death. Perhaps autophagy inhibition could be coupled with crippled pH regulation to foster increased tumor cell death in melanoma. While mTOR inhibition alone has a cytostatic effect, inhibition of mTOR in conjunction with autophagy inhibition (HCQ) showed a synergistic cell killing effect in melanoma cells. Sinomenine was able to induce apoptosis and autophagy in B16F10 melanoma cells [15]. Inhibition of PI3K/Akt/mTOR was shown responsible for these effects and chloroquine could partially reverse the effects of sinomenine. Polygonatum

Correspondence to: James L Cox, Department of Biochemistry, AT Still University, Kirksville, Missouri, USA, Tel: 6606262466; E-mail: jcox@atsu.edu

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cyrtonema lectin induces both apoptosis and autophagic cell death in A375 melanoma cells [16]. A ROS-mediated p38-p53 pathway was shown to be involved in the apoptosis and autophagy implying a connection between the two pathways. Treatment with 3-MA increased survival of melanoma cells when treated with the lectin. Xie et al. showed autophagy dependence of autophagy by knocking down ATG7 expression, resulting in cell death [17]. An inhibitor of mTOR, temsirolimus, induces autophagy in melanoma cells. When coupled with an autophagy inhibitor, CHQ, temsirolimus induced apoptosis and hence might be a way to join mTOR inhibition to cell death induction in melanoma.

ER stress induction due to BRAF activation also activates autophagy due to JNK activation of Beclin 1 [4]. Re-sensitization to chemotherapy may be possible through inhibition of ER stress and attendant autophagy as both are potent survival pathways. Melanoma cells have a strict dependence for the essential amino acid leucine [18]. Low leucine levels do not stimulate autophagy due to Ras-MEK signaling. MEK blockers induced autophagy under conditions of leucine deprivation. A combination of an autophagy inhibitor and leucine-free diet synergistically blocked growth of human melanoma tumors. Treatment of melanoma with Δ^9 -tetrahydrocannabinol (THC) activated autophagy and induced apoptosis [19]. Interesting was the finding that the non-canonical autophagy induction could promote caspase independent cell death and apoptosis.

Inhibition of autophagy in melanoma increased infiltration of natural killer cells (NK) into tumors [20]. The NK cells were fully functional and could account for tumor size reduction of B16 melanoma tumors. An increase of chemokine CCL5 was mainly responsible for increased NK cell infiltration upon autophagy inhibition.

Increase in cell stress with BRAFⁱ activates stress response factor ATF4 that can stimulate LC3 production and hence increase autophagy. Chemical chaperones reduce BRAFV600E mediated ER stress thereby reducing autophagy and increase melanoma cell sensitivity to apoptosis [4]. Therapies that attenuate ER stress may be effective in combination with other agents that target BRAF mutant melanomas. Newer autophagy inhibitors are being developed which will sensitize melanoma cell killing by tumor targeted agents like vemurafinib [21]. For melanoma, autophagy plays a protective role for oxidative stress induced cell death [22]. Hypoxia in melanoma increases ROS, apoptosis, and autophagy. An autophagy inhibitor increased apoptosis following hypoxia induced ROS increases. Glucose starvation induces autophagic cell death in low metastatic B16F1 melanoma cells [23]. In B16 melanoma, overexpression of miR-290-295, which targets autophagic proteins, confers resistance to glucose starvation induced death. Interestingly, overexpression of miR-290-295 did not compromise B16 melanoma cell survival to other cell stress conditions.

CONCLUSION

While autophagic inhibitors may benefit certain melanoma patients more than others, it is still difficult to monitor tumor autophagy levels in practice. Newer autophagy markers are

needed for rapid determination of melanoma autophagy levels. Modulation of autophagy in melanoma (and other cancer types) may reduce resistance and enhance current anticancer treatments. This is a rapidly developing area. New strategies that involve melanoma treatment through modulation of autophagy are urgently needed and current treatment strategies can be augmented for better results.

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