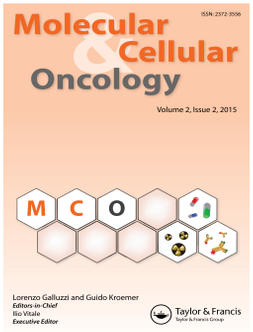

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Fateful music from a talented orchestra with a wicked conductor: Connection between oncogenic BRAF, ER stress, and autophagy in human melanoma

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Abbreviations: ASK1, apoptosis signal-regulating kinase 1; ATF, activating transcription factor; BRAF, serine/threonine-protein kinase B-raf; BCL-2, B-cell lymphoma 2; BCL-XL, BCL2-like 1 isoform 1; BECN1, BECLIN 1; eIF, eukaryotic translation initiation factor; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; GRP78, glucose-regulated protein 78kDa; IRE1, inositol-requiring enzyme 1; JNK, c-Jun N-terminal kinase; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; p38, mitogen-activated protein kinase 14; PERK, double-stranded RNA activated protein kinase (PKR)-like endoplasmic reticulum kinase; TBR3, mammalian homolog of *Drosophila* tribbles; TRAF2, TNF receptor-associated factor 2; UPR, unfolded protein response; 4PBA, 4-phenylbutyric acid.

Autophagy and endoplasmic reticulum (ER) stress are involved in the development, progression, and chemoresistance of melanoma. We recently reported that oncogenic serine/threonine-protein kinase BRAF induces chronic ER stress, hence increasing baseline autophagy and promoting chemoresistance. The attenuation of ER stress restores basal autophagic activity and resensitizes melanoma cells to apoptosis.

Proliferation and cell death are finely regulated and evolutionarily conserved physiologic processes that work in contraposition to play a pivotal role in the homeostasis of a multicellular organism. Indeed, in every organism several million cells are thought to die every second and this phenomenon of cellular demise is balanced by cell proliferation. This epic battle represents a fundamental process throughout the lifespan of an organism, since an unbalanced equilibrium between cell proliferation and death may result in tumor development.

Importantly, most tumors are characterized by an acquired desensitization to apoptotic stimuli, mainly due to dysfunctional apoptosis or deregulated proliferation, as a result of mutations, ablations, or dysfunction of key genes of the 2 signaling pathways.

Moreover, dysfunction or improper activation or inhibition of other molecular pathways may contribute to both tumor growth and unresponsiveness of cancer cells to therapy. Recent studies point to dysregulation of the unfolded protein response (UPR) and autophagy during tumor development/progression and/or under chemotherapeutic treatment that is associated with poor clinical outcome. Indeed, improper activity of these pathways negatively affects drug-induced apoptosis.[1,2]

Both endoplasmic reticulum (ER) stress and autophagy are primarily prosurvival pathways that are activated under stress conditions such as nutrient shortage and hypoxia, a typical feature of the solid tumor microenvironment, and under chemotherapy to actively counteract proapoptotic stimuli. Thus, although targeting ER stress and/or autophagy may provide a considerable benefit for cancer therapy, such potential is complicated by the interconnection of the 2 processes since each can regulate the other. Moreover, in this context the specific function of both ER stress and autophagy is strictly dependent on both tumor type and disease stage, as demonstrated in melanoma.[3]

Cutaneous metastatic melanoma is one of the most aggressive and difficult to treat forms of human malignancy, and moreover has an increasing incidence. Although mutation of the serine/threonine-protein kinase B-raf (BRAF), resulting in

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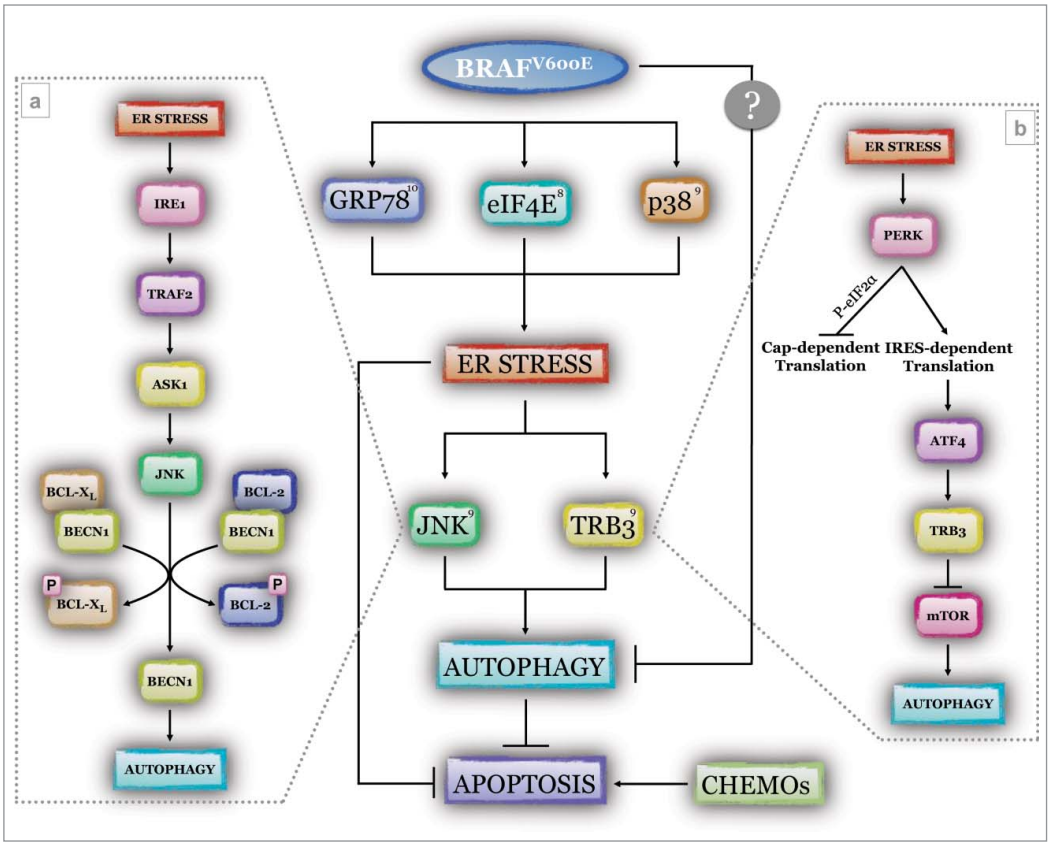

Figure 1. ER stress induced by oncogenic BRAF and modulation of basal autophagy in melanoma. Oncogenic serine/threonine-protein kinase B-raf carrying the V>E mutation (BRAF^{V600E}) stimulates endoplasmic reticulum (ER) stress through the involvement of at least 3 major factors, glucose-regulated protein 78kDa (GRP78), eukaryotic translation initiation factor 4E (eIF4E), and mitogen-activated protein kinase 14 (p38). Through direct interaction with GRP78, mutant BRAF relieves its inhibitory activity toward the 3 stress sensors, activating transcription factor 6 (ATF6), inositol-requiring enzyme (IRE1), and double-stranded RNA activated protein kinase (PKR)-like endoplasmic reticulum kinase (PERK),[10] and thus inducing the unfolded protein response (UPR). Stimulation of the activity of the mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (MEK/ERK) signaling pathway drives phosphorylation of the translational factor eIF4E, hyperstimulating the translational signaling pathway and resulting in protein accumulation in the ER compartment;[8] activation of p38 (through protein phosphorylation) drives the activation of the above mentioned ER stress sensors, resulting in UPR.[9] ER stress in turn enhances the activity of the autophagic process through the PERK/activating transcription factor 4 (ATF4)/mammalian homolog of *Drosophila* tribbles (TRB3) and the IRE1/TNF receptor-associated factor 2 (TRAF2)/apoptosis signal-regulating kinase 1 (ASK1)/c-Jun N-terminal kinase (JNK) axes. In the first signaling pathway (**B**), active PERK phosphorylates the eukaryotic translation initiation factor 2α (eIF2α), a factor controlling the initiation of cap-dependent protein translation, thus stimulating the IRES-dependent translational process. This favors accumulation of the transcription factor ATF4 that in turn stimulates the synthesis of TRB3, which induces the autophagic process through inhibition of mammalian target of rapamycin (mTOR) activity. In parallel (**A**), active IRE1 recruits and activates the cytosolic kinase ASK1 through the adapter molecule TRAF2, which in turn activates JNK. The latter phosphorylates the BECLIN 1 (BECN1) inhibitory partners B-cell lymphoma 2 (BCL-2) and BCL2-like 1 isoform 1 (BCL-X$_L$), resulting in BECN1 release and subsequent autophagy induction. Therefore, mutant BRAF-mediated dysregulation of prosurvival pathways such as ER stress and autophagy sustains tumor development and progression, and desensitizes cancer cells to therapy-induced apoptosis (CHEMOs).

constitutive kinase activation, represents the most characteristic feature of this tumor (present in 70% of all melanomas), other mutations are also involved. Moreover, many reports indicate that dysregulation of autophagy and ER stress may represent 2 key signaling pathways involved in melanoma development, progression, and resistance to therapy, although an univocal interpretation is still far from evident.[4,5] Moreover, the underlying genotype of an individual melanoma also appears to influence UPR and autophagic functions, with BRAF in particular appearing to have a direct effect on both pathways.[6,7] Indeed, oncogenic BRAF reduces cell susceptibility to ER stress-induced apoptosis and concomitantly results in higher basal autophagic rates compared to BRAF wild type melanoma cells, although the underlying molecular mechanisms mediating this effect remain unclear.[6,7] Recently, we and 2 other research groups independently identified 3 molecular pathways linking oncogenic BRAF activity and UPR induction in melanoma cells; these pathways involve (i) eukaryotic translation initiation factor 4E (eIF4E),[8] (ii) mitogen-activated protein kinase 14 (MAPK14, also known as p38)[9] or (iii) glucose-regulated protein 78kDa (GRP78)[10]. Inhibition of eIF4E phosphorylation as a result of oncogenic BRAF-dependent mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (MEK/ERK) activation results in protein hyperproduction and consequent UPR induction.[8] Oncogenic BRAF also results in constitutive p38 activation, contributing to ER stress sensor activation and UPR tuning.[9] Finally, mutated BRAF may also directly interact with GRP78, thus relieving its inhibitory activity on activating transcription factor 6 (ATF6), inositol-requiring enzyme 1 (IRE1), and double-stranded RNA activated protein kinase (PKR)-like endoplasmic reticulum kinase (PERK) and stimulating the UPR signaling pathway[10] (**Fig. 1**).

ER stress activation *per se* enhances tumor growth and inhibits cell death induction,[1] thus promoting tumor progression and chemoresistance. Moreover, oncogenic BRAF-induced UPR also results in autophagy enhancement in

melanoma cells, contributing to desensitization of cells to apoptosis induction.[9,10] Importantly, we have revealed the underlying molecular mechanisms linking the 2 pathways in BRAF mutant melanoma cells based on activation of (i) the PERK/activating transcription factor 4 (ATF4) axis of UPR, resulting in upregulation of mammalian homolog of *Drosophila* tribbles (TRB3) and subsequent inhibition of mammalian target of rapamycin (mTOR) and consequent autophagy induction; ii) the IRE1/TNF receptor-associated factor 2/apoptosis signal-regulating kinase 1/c-Jun N-terminal kinase (IRE1/TRAF2/ASK1/JNK) axis, thus resulting in phosphorylation of B-cell lymphoma 2 (BCL-2)/BCL2-like 1 isoform 1 (BCL-XL), BECLIN 1 (BECN1) release, and autophagy induction[9] (**Fig. 1**).

Inhibition of BRAF-mediated UPR using the chemical chaperone 4-phenylbutyric acid (4PBA) clearly resulted in basal autophagy inhibition and, crucially, resensitization to chemotherapy-stimulated melanoma cell death.[9]

Collectively, these data indicate that, although autophagy dysregulation undoubtedly has a major impact in melanoma, oncogenic BRAF-stimulated UPR assumes a pivotal role in melanoma tumor progression and acquired chemoresistance and, furthermore, directly controls the autophagic process.

In conclusion, targeted therapies that attenuate ER stress may represent a novel and effective therapeutic strategy for melanoma with oncogenic BRAF.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed

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