DRUG RESISTANCE IN MELANOMA

New insights into mechanisms of drug resistance in melanoma

Advanced melanomas are typically resistant to traditional cytotoxic chemotherapies or rapidly develop resistance over time. Recent studies suggest that adaptation to endoplasmic reticulum (ER) stress is a key driver of melanoma progression and resistance to chemotherapy.¹ Peter Hersey, of Newcastle University Calvary Mater at Newcastle, Australia, described advancements in understanding the mechanisms of drug resistance in melanoma, with particular focuses on adaptive processes to ER stress. The hope is that improved understanding of drug resistance mechanisms will ultimately lead to more effective strategies to overcome resistance or prevent its occurrence.

ER stress presumably occurs in melanoma cells due to increased metabolic demands associated with its malignant state. Importantly, Dr. Hersey said, this ER stress is associated with a number of changes and adaptations in melanoma cells that help the cells to survive the stress by increasing resistance to apoptosis. Although there are a number of possible causes of drug resistance in melanoma, adaptive processes to ER stress are arguably the most important. Some of the adaptations resulting in antiapoptotic or other effects linked with melanoma progression and drug resistance include upregulation of the antiapoptotic proteins Mcl-1 and Bcl-XL (but not Bcl-2), activation of the PI3k/Akt/mTOR and RAF/MEK/ERK signaling pathways, upregulation of GRP78 chaperone protein, downregulation of p53, and a switch from the Krebs cycle to glycolysis for glucose metabolism, with consequent acidification of the microenvironment.¹

A key point of Dr. Hersey’s presentation was that taxane- and platinum-based anticancer treatments induce ER-, p53-, and microtubule-dependent apoptosis mechanisms, but they also induce antiapoptotic mechanisms, and it is the balance of the 2 mechanism that is critical and that commonly leads to drug resistance. That is, most treatments induce both cell death and survival pathways, and understanding the survival pathway is as important as understanding the death pathway. Judicious combination of compounds attacking the survival pathway with cytotoxic chemotherapies may be expected to diminish resistance and improve efficacy.

For example, docetaxel-mediated cytotoxicity in melanoma is inhibited by its concurrent activation of the RAF/MEK/ERK pathway, which is linked with class III β-tubulin class levels and activation of protein kinase C-epsilon (PKCe).² This suggests that docetaxel efficacy may be enhanced by coadministration with RAF or MEK inhibitors or PKCe inhibitors. Similarly, resistance to cisplatin in melanoma is related to ER stress-induced GRP78, indicating that its efficacy might be improved if used in combination with GRP78 inhibitors.³ Furthermore, pretreatment with proton pump inhibitors might be expected to reduce resistance to cytotoxic drugs by inhibiting the acidification of the tumor microenvironment that commonly occurs in melanoma.⁶

Plausible arguments can be made for combining cytotoxic chemotherapies with a variety of other agents that target ER stress-induced resistance to apoptosis, including inhibitors of Mcl-1 and agents that target HDM2 and increase p53, among others.¹ Dr. Hersey concluded by suggesting we may already have all the necessary drugs to effectively treat melanoma, and insights from studies on ER stress may provide the basis for more effective use of existing agents.
Explorations of novel targets in melanoma to enhance drug effectiveness

Interferon (IFN)-α2b produces its clinical effects by activating signaling pathways leading to the expression of IFN-stimulated genes (ISGs) and their corresponding proteins, which subsequently act to inhibit cell proliferation and promote apoptosis. Epigenetic silencing of the IFN response system via methylation of the promoter region of ISGs has been proposed as a mechanism underlying the resistance of melanoma cells to IFN-α2b and other antitumor agents.\(^1\) \textbf{Ernest Borden}, of the Cleveland Clinic Foundation in Cleveland, Ohio, discussed the possibility of using compounds that reverse epigenetic silencing of ISGs as a means to enhance the efficacy of IFN-α2b, and possibly other immunomodulators and cytotoxics as well.

Dr. Borden described the results from a study that exposed A375 melanoma cells to 5-Aza-dC (5-Aza-2′-deoxycytidine) prior to treatment with IFN-α2b or IFN-β.\(^2\) A375 melanoma cells are characterized by resistance to the apoptosis-inducing effects of IFN-α2b or IFN-β, and 5-Aza-dC is a DNA demethylating nucleoside analog that works by inhibiting DNA methyltransferase 1 (DNMT1).\(^1\) 5-Aza-dC pretreatment overcame the characteristic resistance of A375 cells to apoptosis induction by IFNs and was associated with 10-fold augmented expression of ISGs.\(^2\) Similar enhancement of IFN-related responses was observed when the cells were depleted of DNMT1 via treatment with an antisense to the enzyme.

Dr. Borden noted that these results were essentially replicated in another study employing 5-Aza-dC or antisense DNMT1 pretreatment to reverse the resistance of A375 melanoma cells to IFN-induced apoptosis,\(^3\) and in a third study demonstrating reversal of resistance with 5-Aza-dC in 2 other melanoma cell lines resistant to IFN-induced apoptosis (SK-MEL-3 and SK-MEL-28).\(^4\) Pretreatment with 5-Aza-dC has been demonstrated to increase expression of a number of proapoptotic proteins in IFN-resistant cell lines, including RASSF1A, XIAP, and TRAIL-R1.\(^1,4\)

Taken together, Dr. Borden, concluded, these results suggest that hypermethylation of ISGs underlies the resistance of at least some melanoma cells to IFN therapy, and that such resistance may be overcome through the use of DNA demethylating agents. Other mechanisms of resistance more briefly mentioned by Dr. Borden included induction of DNA repair molecules, which interfere with the mechanism of action of cytotoxic alkylating agents, and elevation of protein tyrosine phosphatases (PTP), which disrupt cytokine and
IFN signaling. Methoxyamine (and/or related agents) may be used to potentiate cytotoxic chemotherapy, while phosphatase inhibitors (such as SHP-1 and MKP-1) may be used to augment cytokines, chemotherapeutic or immunotherapy, and immune effector cells.

References
4. Bae SI, Cheriyath V, Jacobs BS, Reu FJ, Borden EC. Reversal of methylation silencing of Apo2L/TRAIL receptor 1 (DR4) expression overcomes resistance of SK-MEL-3 and SK-MEL-28 melanoma cells to interferons (IFNs) or Apo2L/TRAIL. *Oncogene.* 2008;27:490-498.

Innovative models to determine chemoresistance
Advanced melanomas are notoriously resistant to cytotoxic chemotherapy. Claus Garbe of Eberhard-Karls University in Tuebingen, Germany, discussed research to study the mechanisms of chemoresistance in melanoma and possible approaches to overcome the resistance. Dr. Garbe began by outlining some expectations for research models used to study chemoresistance: an ability to provide exact measurements and quantitation of cell proliferation and apoptosis, assays that provide with good reproducibility and that are sufficiently simple to perform, potential to assess signaling, models allowing investigation of tumor growth in its natural environment, and animal models that provide for study of the processes in vivo, including animal imaging.

Currently available models involve either cell culture (single-layer cultures of established tumor cell lines or of freshly grown tumor cells, spheroid cell cultures, or organotypic cell cultures) or animal models (embryonic chicken model and syn- or allogeneic mouse models. Single layer cell cultures (ie, monolayer cultures) enable measurement of cell proliferation and apoptosis, and signaling processes, while organotypic cultures (eg, 3-dimensional model in skin reconstructs) can be used to examine invasion, proliferation, and apoptosis within tissue formation. The embryonic chicken model enables measurement of invasion, proliferation, and epithelial mesenchymal transition, and mouse models have been used to measure tumor proliferation, metastasis, and for imaging studies.

By way of example, Dr. Garbe cited work by his own group led by F. Meier, which used monolayer cultures of metastatic melanoma cell lines to demonstrate that dual blockade of RAS/RAF/MEK/ERK (MAPK) signaling (with sorafenib) and PI3K/AKT (AKT) signaling (with wortmannin or LY294002) produced greater inhibition of melanoma cell growth and greater induction of apoptosis compared with blockage of only 1 signaling pathway. The group also used an organotypic skin culture to study the impact of MAPK and/or AKT blockade in a more physiological cultures, showing greater suppression of invasive melanoma growth with dual inhibition of MAPK and AKT versus inhibition of either signaling pathway alone.

Combined inhibition of MAPK signaling with sorafenib and mTOR signaling with rapamycin (sirolimus) has also been shown to enhance apoptosis and inhibition of melanoma cell growth in monolayer
cultures of metastatic melanoma cells, compared with inhibition of either pathway alone, and completely suppresses invasive melanoma growth in organotypic skin culture of human skin and melanoma. Similarly, preliminary results from another study by F. Meier and associates using metastatic melanoma cell lines suggest combining temozolomide with the PI3K inhibitor LY294002 or the mTOR inhibitor rapamycin renders melanoma cells more susceptible to the cytotoxic effects of temozolomide.

A fourth study cited by Dr. Garbe used melanoma cell lines from different stages of progression in monolayer and organotypic skin culture to show that increased expression of the adhesion molecule L1 (CD171) appears to drive melanoma progression, including conversion from radial to vertical phase and enhancing melanoma cell migration and invasion. This illustrates how cell cultures can be utilized to advance knowledge of the pathophysiology of melanoma, thereby suggesting possible new therapeutic targets. In vivo models, such as the embryonic chicken model, have also been utilized to increase understanding of the biology and pathophysiology of melanoma, with 1 study implicating overexpression of bone morphogenetic protein-2 (BMP-2) in invasive melanoma growth, and suggesting the BMP-2 antagonist noggin may used to inhibit such invasion. Dr. Garbe noted that mouse genetic models are being used to try and better understand the genetics of melanoma, although the utility of such approaches warrants further demonstration in the future.

References