Dangerous liaisons: flirtations between oncogenic BRAF and GRP78 in drug-resistant melanomas

Shirish Shenolikar


**Commentary**

*BRAF* mutations in aggressive melanomas result in kinase activation. BRAF inhibitors reduce *BRAF*<sup>V600E</sup> tumors, but rapid resistance follows. In this issue of the *JCI*, Ma and colleagues report that vemurafenib activates ER stress and autophagy in *BRAF*<sup>V600E</sup> melanoma cells, through sequestration of the ER chaperone GRP78 by the mutant BRAF and subsequent PERK activation. In preclinical studies, treating vemurafenib-resistant melanoma with a combination of vemurafenib and an autophagy inhibitor reduced tumor load. Further work is needed to establish clinical relevance of this resistance mechanism and demonstrate efficacy of autophagy and kinase inhibitor combinations in melanoma treatment.

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Protein kinases and cancer
Molecular analyses of human tumors have highlighted the numerous mutations in protein kinase genes that contribute to the development of cancers. For example, the gene encoding BRAF kinase is mutated in more than 60% of melanomas, the most aggressive human skin cancer. Indeed, a mutation that results in a single amino acid substitution, V600E, accounts for 90% of identified BRAF mutations. It is worth noting that this “oncogenic” BRAF (BRAFV600E) is present in many melanocytic nevi, which are benign neoplasias that can persist for decades without transitioning to malignancy (1). The fact that BRAFV600E-containing melanocytic nevi are not intrinsically aggressive hints that additional steps — such as acquisition of further mutations, metabolic reprogramming (2), or alterations in other cellular processes — are required for conversion of the proliferative melanocytes into full-fledged malignant melanomas. This raises the exciting possibility that understanding some of the internal brakes on malignancy will yield novel treatments for melanoma that fails to respond to most current anticancer therapies.

Cancer-promoting mutations frequently result in constitutive activation of the mutant kinase, which has prompted pharmaceutical companies to develop kinase inhibitors to slow or reverse the oncogenic process (3). However, the networks of signaling pathways that control cell growth in normal and cancer cells means that most kinase inhibitors are cytostatic, causing cell cycle arrest rather than cell death. Some kinase inhibitors, however, show spectacular results in eradicating tumor cells. For example, the BCR-ABL tyrosine kinase inhibitor ima-

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Conflict of interest: The author’s research is supported in part by an Academic Center of Excellence (ACE) Award from GlaxoSmithKline Inc.

Citation for this article: J Clin Invest. 2014;124(3):973–976. doi:10.1172/JCI74609.
tinib elicits greater than 80% response in the chronic phase of chronic myeloid leukemia (CML). Similarly, the BRAF inhibitor vemurafenib shows dramatic responses in melanoma patients harboring the BRAFV600E mutation (4). For both imatinib and vemurafenib, drug resistance emerges rapidly in nearly all patients. Understanding how these drugs instigate cell death and the molecular basis underlying resistance should facilitate future development of improved anticancer therapies.

**Kinase inhibitors activate ER stress and induce autophagy**

The combination of an unfavorable microenvironment, a poor nutrient supply, and the energy demands associated with increased protein synthesis in rapidly proliferating cancer cells create ER stress. In melanoma cells, ER stress is reflected by elevated expression of the ER luminal marker glucose-regulated protein 78 (GRP78; also known as BIP) (Figure 1 and ref. 5). Additionally, there is growing awareness that many anticancer therapies induce ER stress, which plays a key role in triggering apoptosis, or programmed cell death, in tumor cells. For instance, vemurafenib has been shown to activate ER stress signaling pathways in melanoma cells, specifically those triggered by the ER membrane–localized sensors PERK, ATF6, and IRE1. In BRAFV600E melanoma cells, knockdown of ATF4, an ER stress–induced transcription factor acting in the PERK pathway, markedly reduced vemurafenib–associated cell death. Conversely, vemurafenib combined with either thapsigargin or tunicamycin, both of which are known inducers of ER stress, enhanced apoptosis in vemurafenib–resistant or -insensitive melanoma cells (6). These results suggest that pharmacological enhancers of ER stress, particularly those that stimulate the PERK pathway, may be useful adjuncts to vemurafenib treatment and may overcome the rapidly developing drug resistance seen in melanoma patients.

The transient activation of the PERK, ATF6, and IRE1 ER stress–activated pathways constitutes a normal homeostatic mechanism present in all mammalian cells to cope with unfavorable environmental conditions. In contrast, persistent signaling via the PERK (and IRE1) pathway promotes apoptosis, eliminating damaged or dysfunctional cells and preserving overall tissue function. The PERK pathway also stimulates autophagy to salvage components of the damaged cells that can be used to sustain the remaining cells (Figure 1 and ref. 5). In a cancer setting, autophagy frequently overrides or overcomes ER stress–induced cell death; therefore, therapy-induced ER stress, which is frequently accompanied...
by enhanced autophagy, may contribute to the development of drug resistance. In CML cells, attenuating autophagy in combination with imatinib therapy enhances cell death and even eliminates CML stem cells, which are often difficult to eradicate with imatinib alone (7). In this issue of the JCI, Ma et al. reveal that autophagy is also elevated in melanoma tumor samples from patients treated with the BRAF inhibitors vemurafenib and dabrafenib (8). Autophagy biomarkers were also increased in samples taken after the emergence of drug resistance compared with samples taken prior to treatment. Furthermore, BRAF inhibitor–induced autophagy was preceded by ER stress in both drug-sensitive and -resistant BRAFV600E melanoma cell lines. Importantly, autophagy inhibited kinase inhibitor–induced cell death both in culture and in a mouse xenograft melanoma model. Together, these findings raise new hopes that a similar drug combination strategy may limit drug resistance and improve the efficacy of current treatment regimens for malignant melanoma.

The work by Ma and colleagues (8) also provides mechanistic insight into the actions of BRAF inhibitors, suggesting that they act upstream of PERK and promote PERK autophosphorylation, which is canonically activated in response to misfolded proteins in the ER. Misfolded proteins displace GRP78, which suppresses PERK kinase activity by binding its ER luminal domain. GRP78 displacement promotes PERK dimerization and autophosphorylation, increasing PERK activity, as evidenced by the enhanced phosphorylation of the eukaryotic initiation factor eIF2α and the subsequent attenuation of mRNA translation. Remarkably, Ma and colleagues found that BRAF inhibitors enhanced GRP78 binding to BRAFV600E within the ER lumen, and this interaction correlated with a reduction in GRP78 binding to PERK (8). These data suggest that in the presence of BRAF inhibitors, BRAFV600E outcompetes PERK for GRP78 binding, thereby explaining the activation of PERK by these drugs. The interaction between GRP78 and BRAFV600E is somewhat surprising, because GRP78 is a well-recognized ER luminal chaperone and possesses a C-terminal KDEL ER retention sequence. However, some studies have suggested that GRP78 can be translocated to the cytosol and plasma membrane in response to ER stress (9). In contrast, BRAF is primarily cytoplasmic, although one report suggests that a small fraction of BRAF in thyroid cancer cells may be associated with mitochondria. To date, the ER localization of BRAF — either WT or mutant forms of the protein — has not been reported. Substantially less BRAFV600E bound to GRP78 in the absence of BRAF inhibitors in the cytoplasm.

Conclusions and future directions

An interpretation of the work of Ma et al. (8) might be that BRAF inhibitors promote the translocation of BRAFV600E to ER, a major site of GRP78 in cells, thereby enhancing the BRAF/GRP78 interaction. A puzzling aspect is the apparent finding that PERK was preferentially or selectively activated after GRP78 binding to BRAF. The ER luminal domains of the three ER stress sensors, PERK, ATF6, and IRE1, are highly homologous, and all three proteins bind GRP78; therefore, it would be expected that the sequestration of GRP78 by BRAFV600E might activate all three ER stress–signaling pathways. On the other hand, earlier studies have shown that GRP78, which is induced by ATF6, is decreased in melanoma cells after vemurafenib exposure (6). At this stage, not only is it unclear how BRAF inhibitors promote BRAF entry into the ER, but it also remains to be determined how and why the sequestration of GRP78 by BRAFV600E might activate all three ER stress–signaling pathways. An emerging complicating factor is the recent finding that vemurafenib displaces BRAFV600E from the HSP90/CDC37 chaperone/cochaperone complex (10). The displaced BRAFV600E is unstable and is degraded by the proteasome, which suggests that this kinase inhibitor achieves some of its therapeutic effect by reducing cellular BRAFV600E levels. Whereas these data indicate that vemurafenib decreases both GRP78 and BRAFV600E levels in melanoma cells, Ma and colleagues demonstrated an enhanced association of GRP78 and BRAFV600E in the presence of this BRAF inhibitor (8). Thus, more work is clearly needed to fully understand these differing effects of the BRAF inhibitor and their impact on ER stress in vemurafenib-treated cells.

In summary, recent studies highlight the ability of anticancer therapies to activate ER stress, which may be critical for the induction of apoptosis in tumor cells; however, therapy-induced ER stress, specifically that associated with PERK activation, also promotes cancer cell survival via activation of autophagy (11). Other studies suggest that the PERK pathway also enhances the expression of inhibitors of apoptosis (IAPs), which are frequently elevated in melanoma cells and may also contribute to cancer cell survival or drug resistance. An emerging strategy for a more effective anticancer therapy is to combine kinase inhibitors with autophagy inhibitors to achieve the best clinical outcomes for CML and melanoma. The inclusion of IAP-inhibiting compounds to enhance apoptosis may also help reduce the rapid resistance to the frontline drugs. It will be exciting to see just how this new strategy plays out in the coming years and whether it offers new hope for the eradication of the aforementioned cancers.

Acknowledgments

The author’s research is supported by grants from National Medical Research Council and Biomedical Research Council of Singapore as well as by a Duke/Duke-NUS Collaborative Research Grant. Additional funding was provided through a GlaxoSmithKline Academic Center of Excellence (ACE) Award.

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Dynamin 2 the rescue for centronuclear myopathy

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Centronuclear myopathy (CNM) is an inherited muscle-wasting disease with onset in infancy or childhood. Muscle biopsies from CNM patients characteristically show enlarged and centrally placed nuclei throughout the muscle, and clinically, CNM-associated mutations may result in weakened respiratory musculature, leading to early death without supportive care. Three main forms of CNM are attributable to mutations in myotubularin (MTM1), amphiphysin 2 (AMPH2), and dynamin 2 (DNM2), which all encode membrane-associated proteins that potentially act in membranes critical for excitation-contraction coupling. XLCNM is the result of mutations in MTM1, which encodes a ubiquitously expressed phosphoinositide 3-phosphatase that functions to dephosphorylate PI3P and phosphatidylinositol-3,4-bisphosphate, two phospholipids essential for membrane sorting and signaling (2). MTM1 is required for normal endocytosis, receptor degradation, and early endosome maturation (1).

Mice harboring a deletion of Mtm1 (Mtm1−/y mice) recapitulate human disease and display many characteristic XLCNM-associated features, including muscle weakness, centralized nuclei, and muscle atrophy (3). Mtm1−/y mice exhibit muscle atrophy, become weak at 3 to 4 weeks of age, and have a greatly reduced life expectancy of only 6 to 12 weeks (3). The role of MTM1 in human muscle has also been effectively modeled in zebrafish using morpholinos to reduce mtm1 expression (MTM MO) (4). MTM MO fish, like XLCNM patients and Mtm1−/y mice, develop weakness and muscle atrophy. MTM MO fish display accumulation of PI3P, especially surrounding muscle nuclei, and T-tubule defects. The phenotypes observed in MTM MO fish provide further support that MTM1 is critical for the normal biogenesis and maintenance of membrane structures within muscle (4). Because of its broad role as a lipid phosphatase, the indispensable targets of MTM1’s enzymatic action are not fully known.

DNM2 is a ubiquitously expressed GTPase that has been implicated in multiple cellular functions, including endocytosis, membrane scission, and cytoskeletal remodeling (5). Dynamins assemble as rings around membrane tubules where they are thought to actively “pinch off” membranes (5). Complete loss of Dnm2 results in embryonic lethality in mice (6). In humans, CNM is caused by dominant DNM2 mutations (7), and overexpression of a CNM-linked DNM2 mutation (R465W) in mouse muscle results in myopathic features, including centralized nuclei, muscle atrophy, and deformed T-tubules (8). Interestingly, overexpression of normal DNM2 in mouse models also produces some of these same features, consistent with the model that increased DNM2 activity contributes to CNM (8). Increased DNM2 activity may lead to excessive membrane scission and pruning, giving the appearance of excessive membrane accumulation around nuclei or at T-tubules.

Genetic reduction of DNM2 in a CNM model

In this issue of the JCI, Cowling and colleagues described elevated protein levels of DNM2 in Mtm1−/y animals and in muscle biopsies from human XLCNM patients (9). To target the increased levels of DNM2 found in Mtm1−/y animals, mice heterozygous for Dnm2 were crossed with Mtm1−/y mice. The in vivo reduction of Dnm2 corrected some histological abnormalities in muscle, but dramatically extended life expectancy from 6 to 12 weeks to beyond one year. To demonstrate that this effect was muscle intrinsic, Cowling and colleagues generated Mtm1−/y mice in which Dnm2 was specifically reduced in skeletal muscle. Reduction of Dnm2 in skeletal muscle alone after disease onset was sufficient to reduce pathology and...