levels that correlated positively with the hBD-2 levels. These observations suggest a role for a gene–cytokine interaction in the occurrence of dermatophytosis, associating the infection with decreased DEFB4 CN, and elevated serum hBD-2 and IL-22 levels. The authors hypothesize that the absence of a correlation between DEFB4 CN variation and serum hBD-2 levels in patients suggest that the genetic background is the main factor controlling hBD-2 production in the absence of infection, whereas upregulation of expression by *T. rubrum*, likely by stimulating IL-22 production, may cause the higher levels that are seen in patients.

Among other hypotheses, the data presented by Jaradat *et al.* (2014) suggest that low DEFB4 CN may be a risk factor for dermatophytosis, with elevated IL-22 levels implicated in its pathogenesis.

More scientific contributions that provide new knowledge about the genetic and other predisposing factors for dermatophytosis are important because of their implications for prophylaxis and for therapy. Identifying individuals who are susceptible to chronic dermatophytosis would allow for infection and recurrence prevention. According to Jaradat *et al.* (2014), patients with low DEFB4 CN may be predisposed to all dermatophytes, but a more chronic and recalcitrant to treatment subtype like onychomycosis could be prevented by aggressive treatment of tinea pedis in these patients. In addition, identifying high-risk families would allow for education of their members about the risk of fungal infections. New therapeutic strategies can be developed as well to target the altered immune response pathways; considering this article's findings, one potential target that merits exploration is IL-22.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Metabolic Vulnerability in Melanoma: A ME2 (Me Too) Story

Bin Zheng[1] and David E. Fisher[1]

Metabolic reprograming is a hallmark of cancer and might represent an Achilles' heel in cancer cells. The study by Chang *et al.* in this issue highlights a critical role of mitochondrial malic enzyme 2 (ME2) in melanoma progression. Targeting ME2 could be an effective approach to inhibit melanoma cell proliferation and tumor growth.

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Altered cellular metabolism is a hallmark of cancer. To successfully undergo oncogenic transformation, cells must meet the bioenergetic and biosynthetic demands for growth and proliferation. For example, most cancer cells have increased uptake of glucose and glycolytic flux to support the generation of metabolic intermediates, such as amino acids, nucleotides, and fatty acids that are required to build a new cell (Vander Heiden *et al.*, 2009). In addition to glucose, cancer cells often use glutamine as another major nutrient source and metabolize it through glutaminolysis to replenish the tricarboxylic acid (TCA) cycle intermediates that are utilized for various biosynthetic reactions (Vander Heiden *et al.*, 2009). Importantly, expression and activity of key enzymes in these metabolic pathways are regulated by various oncogenes and tumor suppressors, providing a mechanistic explanation for metabolic reprograming in cancer. Understanding the underlying mechanisms will therefore be critical to developing better diagnostic tools and therapeutic strategies for cancer, including melanoma, where metabolic regulation was seen

[1]*Department of Dermatology, Cutaneous Biology Research Center, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts, USA*

Correspondence: David E. Fisher, Department of Dermatology, Cutaneous Biology Research Center, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts 02129, USA. E-mail: dfisher3@mgh.harvard.edu

Clinical Implications

- Expression of mitochondrial malic enzyme (ME2) increases during the progression from melanocytic nevi to malignant melanoma.
- Knockdown of ME2 activates AMP-activated protein kinase and impaired melanoma cell proliferation and tumor growth.
- Small molecule inhibitors of ME2 might be exploited for melanoma therapy.

to interface with BRAF-targeted therapy (Haq *et al.*, 2014).

Malic enzymes (MEs) catalyze the oxidative decarboxylation of malate, a TCA cycle intermediate, to pyruvate, with the concomitant reduction in $NAD(P)^+$ to NAD(P)H. There are three isoforms of MEs in mammals with different cofactor specificity and subcellular localizations: the cytosolic $NADP^+$-dependent ME1, the mitochondrial $NAD(P)^+$-dependent ME2, and the mitochondrial $NADP^+$-dependent ME3. Although the enzymatic activities of MEs have been well characterized *in vitro* (Chang and Tong, 2003), very little is known about their biological roles, especially in the context of cancer biology. In work reported in this issue, Chang *et al.* (2014) examined the expression of all three isoforms of MEs in melanoma using microarray data mining and IHC analysis. The authors observed that both mRNA and protein levels of ME2, but not of the other two isoforms, increase during the progression from melanocytic nevi to malignant melanoma (Chang *et al.*, 2014), suggesting a role of ME2 in melanoma biology. Chang *et al.* (2014) further demonstrated that shRNA-mediated knockdown of ME2 in A375 (BRAF V600E mutant) and A2058 (BRAF WT) melanoma cells attenuated cell proliferation. Moreover, knockdown of ME2 in A2058 cells impaired their ability to grow on soft agar and to develop xenografted tumors in non-obese diabetic/severe combined immunodeficient mice (Chang *et al.*, 2014), suggesting that ME2 is essential for the tumorigenicity of these melanoma cell lines.

In addition to its role in regulating cell proliferation and tumor growth, ME2 may also modulate senescence. Downregulation of ME2 was recently found to induce senescence in IMR90 fibroblasts, whereas expression of ME2 suppressed senescence in these cells (Jiang *et al.*, 2013). In melanocytes, oncogenic BRAF V600E is known to induce senescence, and this process was recently suggested to be associated with an increase in pyruvate entry into the mitochondrial TCA cycle (Kaplon *et al.*, 2013). Intriguingly, enhanced expression of ME2 may have a similar metabolic effect as BRAF V600E on the flux of pyruvate into the TCA cycle, as ME2 converts malate into pyruvate in mitochondria. Although it is tempting to speculate that ME2 could regulate melanocyte senescence, in part because of lower ME2 expression levels in nevi (Chang *et al.*, 2014), this question awaits further investigation.

Using scratch wound healing and matrigel-based invasion assays, Chang *et al.* (2014) demonstrate that knockdown of ME2 inhibited A2058 melanoma cell motility and invasion *in vitro*, whereas ectopic expression of ME2 reversed this phenotype. ME2 expression did not impact metastatic potential or overall survival in the tested cohort of Chang *et al.* (2014), although a recent study (Ren *et al.*, 2014) reported elevated ME2 protein expression in metastatic melanomas compared with primary tumors, based on IHC analysis. Hence, it remains to be seen whether ME2 expression can serve as a biomarker for melanoma metastasis.

In this study, Chang *et al.* (2014) also examined the metabolic consequence of ME2 knockdown in melanoma cells. ME2 may use either NAD or NADP as a cofactor in the oxidative decarboxylation reaction that generates NADH or NAPDH, respectively. Because NADH is the major donor for generating a proton gradient across the mitochondrial membrane, which, in turn, is used to drive ATP production, it is conceivable that ME2 may regulate ATP production. Indeed, knockdown of ME2 in melanoma cells reduced cellular ATP levels (Chang *et al.*, 2014). This effect may, moreover, result from the decreased generation of pyruvate from malate, which may attenuate flux through the TCA cycle. Furthermore, knockdown of ME2 increased reactive oxygen species (ROS) levels in melanoma cells (Chang *et al.*, 2014), possibly because of reducing the availablility of NADPH to scavenge ROS. A reduction in mitochondrial NADPH production from ME2 could lead to an accumulation of mitochondrial ROS. Nonetheless, in-depth metabolic analyses, such as stable isotope labeling and metabolic flux analysis, would provide additional insight into the metabolic role of ME2 in melanoma.

An important finding in this study is the negative regulation of AMP-activated protein kinase (AMPK) activity by ME2 in melanoma. Chang *et al.* (2014)found that knockdown of ME2 led to activation of the AMPK signaling pathway, whereas ectopic expression of ME2 was sufficient to inhibit AMPK. As an evolutionarily conserved energy sensor, AMPK regulates energy homeostasis by monitoring changes in intracellular AMP, ADP, and ATP concentrations (Hardie *et al.*, 2012). Elevated AMP (or ADP) to ATP ratios activate AMPK and trigger it to phosphorylate downstream effectors that stimulate ATP-producing catabolic pathways, while suppressing ATP-consuming biosynthetic pathways, thus maintaining an energy balance (Hardie *et al.*, 2012). In addition to its well-established effect on metabolism, AMPK has been shown to regulate a variety of cellular processes, including suppression of cell growth and proliferation in response to metabolic stress, supporting a potential tumor suppressor role for AMPK. In this regard, it is interesting to note that loss of AMPKα1 expression was recently reported to be associated with poor overall and disease-specific 5-year survival in a cohort of 128 melanoma patients (Bhandaru *et al.*, 2014). The activation of AMPK can be triggered by various metabolic stresses, such as hypoxia, glucose deprivation, and ROS. Thus, the decreased ATP production and/or the increased ROS accumula-

tion from ME2 downregulation observed by Chang *et al.* (2014) could be responsible for the AMPK activation. Previously, the activity of AMPK in melanoma was found to be repressed by BRAF V600E mutant, a major driver of melanoma (Zheng *et al.*, 2009). It would be interesting to determine whether BRAF and ME2 coordinate in attenuating AMPK activity in melanoma. In addition, whether AMPK is indeed a critical mediator of ME2's effects on cell proliferation and tumor growth in melanoma needs to be addressed experimentally.

The critical role of AMPK in suppressing cell growth and proliferation raises the interesting possibility that activation of this pathway would suppress tumor growth. More recently, phenformin, an inhibitor of the mitochondria complex I respiratory chain and an AMPK activator, was shown to enhance the anti-tumor activitiy of BRAF inhibitors in cultured melanoma cells and to promote tumor regression in several xenograft models and a genetically engineered BRAF V600E–driven mouse model of melanoma (Yuan *et al.*, 2013). Phenformin selectively targets a minor subpopulation (approximately 5–10%) of melanoma cells expressing JARID1B, a H3K4 demethylase and marker for slow cycling cells, but it has lower anti-tumor activity on the JARID1B-negative cells (Yuan *et al.*, 2013). Conversely, although BRAF inhibitors are effective in killing the bulk of BRAF mutant melanoma tumors—i.e., JARID1B-negative cells—they do not appear to be effective on JARID1B-positive cells (Yuan *et al.*, 2013). These results suggest that metabolic heterogeneity exists in these tumors, and this may contribute to different states of treatment sensitivity and resistance. The metabolic differences between these two subpopulations of cells are still uncharacterized. It certainly would be interesting to investigate whether ME2 contributes to this metabolic heterogeneity.

In summary, the work by Chang *et al.* (2014) characterizes the function of ME2 in melanoma, providing new insight into metabolic reprograming in melanoma. These findings suggest a previously unknown vulnerability of melanoma cells. If the catalytic activity of ME2, indeed, proves to be critical for the biological roles characterized in this study, small molecule inhibitors of ME2 might be exploited for melanoma therapy.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Unfolding the Mutational Landscape of Human Melanoma

Diwakar Davar[1], Yan Lin[2] and John M. Kirkwood[1]

Over the preceding two decades, sophisticated sequencing techniques have been used to characterize the genetic drivers of adult melanoma. However, our understanding of pediatric melanomas is still rudimentary. In this report, we comment on a thorough multi-platform analysis of common pediatric melanoma subsets, including pediatric conventional melanoma (CM), congenital nevus-derived melanoma (CNM), and Spitzoid melanoma (SM), contributed by Lu *et al.*

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Data from whole-genome and -exome sequencing (WGS/WES) and The Cancer Genome Atlas (TCGA) have revealed multiple new insights to the nature of cutaneous melanoma, etiology, and potential avenues of therapy. First, the role of oral BRAF/MEK inhibitors (based upon the frequency of ∼50% with UV signature activating mutations in *BRAF* and the *MAPK* pathway) has become abundantly apparent. Further, the mutational landscape and high frequency of mutations observed in cutaneous melanoma, which exceeds those of all other

[1]*Department of Medicine, Division of Hematology-Oncology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA and* [2]*Biostatistics Facility, University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania, USA*

Correspondence: John M. Kirkwood, Department of Medicine, Division of Hematology-Oncology, University of Pittsburgh Medical Center, 5150 Centre Avenue, Pittsburgh 15232, Pennsylvania, USA. E-mail: kirkwoodjm@upmc.edu