COMMENTARY

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Change Is in the Air: The Hypoxic Induction of Phenotype Switching in Melanoma

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Melanoma cells can switch from a highly proliferative, less invasive state to a highly invasive, less proliferative state, a phenomenon termed phenotype switching. This results in a highly heterogenous tumor, where a slow-growing, aggressive population of cells may resist tumor therapy, and it predicts tumor recurrence. Here we discuss the observation made by Widmer *et al.* that hypoxia may drive phenotype switching.

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Melanoma is the deadliest form of skin cancer and, although only responsible for 5% of all skin cancer cases, it accounts for over 70% of skin cancer–related deaths. Melanomas display extensive heterogeneity and demonstrate a high degree of phenotypic plasticity. It has been observed that a subpopulation of melanoma cells appears to downregulate signaling pathways associated with proliferation when they migrate (Hoek *et al.*, 2008; Ghislin *et al.*, 2012). This switch from a proliferative phenotype to a migratory or invasive phenotype is achieved by decreases in the expression of genes associated with melanocyte differentiation and pigment production, such as MART1 and GP100, and by increases in genes such as WNT5A (Dissanayake *et al.*, 2008) and FGF2 (Reiland *et al.*, 2006). Melanocyte differentiation antigens such as MART1 and GP100 are regulated by MITF, a critical mediator in the transformation of melanocytes and the growth and proliferation of primary melanomas (Haq and Fisher, 2011). In line with this concept of phenotype switching is the fact that the expression of MITF and its downstream effectors are often decreased in metastatic melanoma (Dissanayake *et al.*, 2008). We do not yet understand completely the factors that promote phenotype switching in melanoma, nor the implications of changes in the microenvironment for the development of this phenotypic plasticity.

In the current issue of *JID*, Widmer *et al.* (2013) demonstrate that one microenvironmental factor that can influence phenotype switching is hypoxia. They demonstrate that in tissue biopsies, melanoma cells that have decreased expression of melanocyte differentiation antigens correlate with hypoxic regions within the tumor, as determined by their expression of GLUT1 and HIF1a. On the basis of the phenotype switching theory, this could imply that the cells within the hypoxic regions are more metastatic. However, the mechanisms are clearly more complex, as hypoxia may be inducing changes leading to cell death in some cells while promoting invasion in others. Either of these opposing outcomes would result in decreased expression of melanocyte differentiation antigens. This complexity is further evidenced by the fact that, in this study, the expression of some genes involved in invasion, and known to be critical for melanoma metastasis *in vitro* and *in vivo* (e.g., Wnt5A), were downregulated in hypoxic cells. Similarly, the expression of some genes involved in proliferation, and previously shown to be downregulated in metastatic melanoma, were elevated. However, of the genes examined, those involved in proliferation were more likely to be downregulated during hypoxia, whereas genes involved in invasion were more likely to be upregulated, suggesting that the hypoxic microenvironment promotes invasion. The authors confirmed this using *in vitro* invasion assays, and other studies have confirmed *in vivo* that hypoxia can increase melanoma metastasis.

A key finding in the Widmer study is that a hypoxic microenvironment can downregulate the expression of MITF. This is in keeping with a study by Cheli *et al.* (2012), which also demonstrated that a hypoxic microenvironment decreases MITF expression and increases tumorigenic properties of melanoma cells. In that study, the authors showed that the downregulation of MITF was mediated through the hypoxia-inducible factor 1α (HIF1α) and the transcription factor Bhlhb2 (basic helix–loop–helix domain containing, class B2). Further, the Cheli study showed that knocking out MITF is sufficient to increase metastatic potential of both mouse and human cells (Cheli *et al.*, 2012). This correlated with an increase in fibronectin and snail, two markers of a more mesenchymal, invasive phenotype. Together, these studies indicate that the hypoxia-induced downregulation of MITF can induce a phenotype switch to a more metastatic phenotype. The Widmer study has also demonstrated that once hypoxia-induced phenotype switching has occurred, returning the cells to normoxic conditions does not reverse the increased invasive potential. This may be due to the upregulation of certain mesenchymal markers such as vimentin, which, once expressed, are difficult to repress.

In addition to demonstrating that hypoxia guides a switch to a more invasive phenotype, via the downregulation of MITF, these data may have significant implications for melanoma

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Clinical Implications

- Hypoxia guides a switch of melanoma cells to a more invasive phenotype, via the downregulation of MITF.
- Thus, the environment in which melanoma cells find themselves may affect their invasive potential.
- Microenvironmental changes such as hypoxia may also facilitate the development of resistance to BRAF inhibitors.

therapy. A recent advancement in therapy for melanoma is the clinical approval of inhibitors of mutant BRAF (BRAFV600E). Mutations in the BRAF oncogene, which signals downstream of NRAS to activate mitogen-activated protein kinase (MAPK) signaling, can be found in over 60% of patients. Although initial responses to the available BRAF inhibitors are usually good, resistance develops quite quickly. Understanding the mechanisms that guide this resistance is a major area of interest in the melanoma field. To date, very few studies have addressed the role of microenvironmental changes such as hypoxia in the development of resistance to BRAF inhibitors. However, an older study demonstrated that mutant BRAF increased HIF1α in melanoma cells via increased MAPK signaling, and that knockdown of BRAF (mutant and wild type) decreased cell survival significantly under hypoxic conditions (Kumar *et al.*, 2007). A very recent study has now shown that BRAF activation suppresses MITF, which in turn affects oxidative phosphorylation in melanoma (Haq *et al.*, 2013). Low levels of MITF corresponded to resistance to BRAF inhibitors. Because tumors that are resistant to BRAF inhibitors have elevated MAPK signaling, it would follow that they have elevated levels of HIF1α and, subsequently, less MITF. Indeed, a previous study from the Dummer laboratory demonstrated that invasive phenotype cells (low MITF) are less susceptible to MAPK inhibition and that exposure of proliferative phenotype cells to BRAF inhibitors results in the expression of invasive phenotype markers (Zipser *et al.*, 2011).

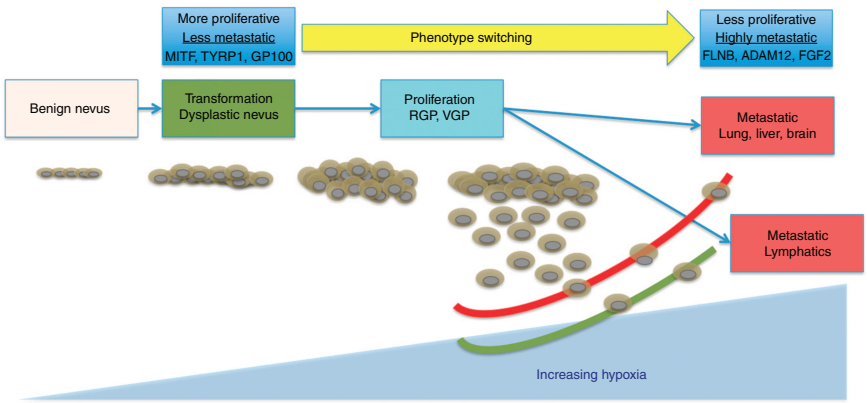

Figure 1. Schematic overview of phenotype switching in melanoma, and the effects of hypoxia thereon. Following transformation of melanocytes, melanoma progresses through the traditional radial and vertical growth phases, where tumor cells eventually come into contact with the blood (red line) or lymphatic system (green). At the point of transformation (green box), a less metastatic and more proliferative phenotype is observed (left blue box). As progression occurs, so does phenotype switching, until a less proliferative highly metastatic gene profile can be seen (right blue box) as tumor cells enter the vertical growth/metastatic phase. This progression can be enhanced further by changes in the tumor microenvironment, such as an increased hypoxic state.

These data, taken together, highlight how mechanisms that guide metastasis might also promote therapy resistance, resulting in a highly aggressive tumor (Figure 1). Clearly, the transcription factor MITF also has a critical role in both these processes. Understanding how hypoxia can guide not only metastasis but also therapy resistance to BRAF inhibitors could be of great value in designing novel therapies for the treatment of melanoma. Targets of MITF may prove, in turn, to be viable targets for enhancing the efficacy of current inhibitors or even as targets of stand-alone therapies.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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