effectiveness and reducing the adverse effects of antimicrobials and other therapeutic agents. Although these applications are promising, many interesting questions remain to be answered and many challenges remain to be overcome. One important issue is the comparability of the results of *in vitro* and *in vivo* studies: the results of *in vitro* studies do not necessarily predict the outcome of *in vivo* exposure. In addition, there are growing concerns regarding the potential toxicities of materials used to fabricate some particles. Additional studies are needed to improve our understanding of this important field now in development.

CONFLICT OF INTEREST

The author states no conflict of interest.

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See related article on pg 1269

Mutant *BRAF*: A Novel Mediator of Microenvironmental Escape in Melanoma?

Keiran S.M. Smalley[1,2]

The acquisition of mutant *BRAF* is an important initiating event for melanoma development, although the process by which transformed melanocytes escape from keratinocyte control and disseminate to other organs is not well understood. Boyd *et al.* (2013) provide evidence that oncogenic *BRAF* contributes to the microenvironmental escape of melanocytes through the downregulation of E-cadherin expression via the transcriptional suppressor Tbx3.

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The earliest stages of melanoma development, in which transformed melanocytes escape the constraints of the local microenvironment and disseminate to lymphatic vessels and distant organs, are still being elucidated. Under normal physiological conditions, melanocytes sit at the basal layer of the epidermis where they interact closely with surrounding keratinocytes at a ratio of about 1:5. Under these circumstances, the two cell types exhibit a close relationship, with melanin pigment (in the form of melanosomes) being actively transported from melanocytes into surrounding keratinocytes. The transfer of melanin to keratinocytes (aka the tanning response) is critical in providing photoprotection to skin and serves to limit the harmful DNA-damaging activity of solar UV radiation (Tran *et al.*, 2008). The process of melanin synthesis and melanosome transport is initiated by signals that emanate from the keratinocytes after the UV-mediated initiation of p53-mediated gene transcription (Tran *et al.*, 2008). This, in turn, leads to the release of α-melanocyte-stimulating hormone from the keratinocytes and the stimulation of melanocortin receptor 1 signaling and melanogenesis in nearby melanocytes. In addition to these events, keratinocytes also control many other aspects of melanocyte behavior, including growth, motility, and differentiation (Haass *et al.*, 2005). This regulation is achieved through a finely balanced signaling network involving direct cell–cell adhesion between melanocytes and keratinocytes, as well as the release of paracrine growth factors. One of the key mediators of melanocyte/keratinocyte interaction is E-cadherin, a calcium-dependent glycoprotein that has important roles in maintaining the cell architecture in epithelial tissues (Haass *et al.*, 2005). Loss of E-cadherin expression is an important step in the majority of epithelial cancers, and it is a prerequisite for dissemination of invasive cells from the initial tumor mass (Kalluri and Weinberg, 2009). Typically, loss of E-cadherin expression is part of a larger dynamic transcriptional program that is frequently observed in cancer cells, called the epithelial-to-mesenchymal transition (EMT). Other features of the EMT include the adoption of a mesenchymal phenotype, increased extracellular matrix deposition and resistance to apoptosis (Kalluri and Weinberg, 2009). Under normal conditions, melanocytes express high levels of E-cadherin (despite being derived from the neural crest) with homotypic E-cadherin-based adhesion

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Clinical Implications

- Under normal conditions, the behavior of melanocytes is partly regulated through E-cadherin-mediated interactions with surrounding keratinocytes.
- The acquisition of *BRAF* mutations downregulates the expression of E-cadherin in melanocytes through the transcriptional repressor Tbx3, increasing their invasive capacity.
- Inhibition of BRAF signaling through either small hairpin RNA knockdown or BRAF inhibitor treatment can partially restore E-cadherin expression.

between melanocytes and keratinocytes, constituting an important homeostatic mechanism in skin. Irrespective of the initiating oncogene, melanoma development is usually associated with decreased E-cadherin expression and an escape from the control of local keratinocytes (Hsu *et al.*, 2000; Haass *et al.*, 2005). Often, the suppression of E-cadherin expression is part of a ''cadherin switch'' that results in a reciprocal increase in the expression of N-cadherin (Haass *et al.*, 2005). This increased expression of N-cadherin contributes to the process of transformation by allowing melanoma cells to switch binding partners and to interact instead with fibroblasts and endothelial cells, as well as through direct signaling effects that increase melanoma survival and invasion (Haass *et al.*, 2005). The importance of E-cadherin downregulation in the development of melanocytic tumors is illustrated by the observation that a reintroduction of E-cadherin brings even aggressive melanoma cells back under keratinocyte control (Hsu *et al.*, 2000). Despite many years of research into the role of E-cadherin downregulation in melanoma development, the mechanisms by which transformed melanocytes downregulate E-cadherin expression and escape from the local environment are still poorly understood.

Melanoma is known to be a diverse group of tumors whose initiation and progression is mediated by distinct oncogenes. By far the most prevalent is *BRAF*, which is known to be mutated in ~50% of all cutaneous melanomas (Smalley, 2010). Although the mechanisms by which mutant *BRAF* mediates oncogenic transformation of melanocytes have been extensively characterized, relatively little is known about its potential role in microenvironmental escape (Smalley, 2010). The study by Boyd *et al.* (2013), published in this issue of the *Journal of Investigative Dermatology*, provides the first evidence that links oncogenic *BRAF* to decreased E-cadherin expression. Using genome-wide transcriptome analysis, the authors demonstrated that introduction of the *BRAF* V600E mutation induced an ''EMT-like'' gene signature in human melanocytes. One of the major hits identified from the screen was E-cadherin, whose expression was found to be suppressed by *BRAF* at both the messenger RNA and protein levels. The BRAF dependency of these effects was demonstrated through small hairpin RNA studies and the ability of the BRAF inhibitor vemurafenib to partially reverse this, leading to increased E-cadherin expression. In epithelial cells, the EMT process is subject to complex regulation by a network of transcription factors such as SLUG, ZEB, Goosecoid, FOXC2, SNAIL, and TWIST, whose expression is controlled partly by growth factors, including transforming growth factor-β, platelet-derived growth factor, epithelial growth factor, and hepatocyte growth factor (HGF) (Kalluri and Weinberg, 2009). Of these, HGF is implicated in the induction of an EMT-like state in human melanocytes, an effect mediated through the increased expression of SNAIL and SLUG, as well as signaling through the PI3K/AKT and mitogen-activated protein kinase (MAPK) signaling pathways (Haass *et al.*, 2005). Despite increased SNAIL expression being associated with decreased E-cadherin expression in melanoma cells, Boyd *et al.* (2013) did not observe any *BRAF*-mediated changes in the expression of known EMT-associated E-cadherin regulators such as Slug, ZEB, TWIST, EZH2, or TCF3. Instead, the introduction of mutant *BRAF* increased the expression and promoter activity of the transcriptional repressor Tbx3. Tbx3 is a T-box family member of development-associated transcription factors implicated in the regulation of cell proliferation, cell fate, and cell identity (Rodriguez *et al.*, 2008; Peres *et al.*, 2010). Overexpression of Tbx3 has been reported in many cancers, including melanoma, where it suppresses entry into senescence through the repression of the cell cycle inhibitors $p14^{ARF}$ and $p21^{CIP1}$ (Peres *et al.*, 2010). Increased Tbx3 expression has been shown to downregulate E-cadherin expression in melanoma cells through direct binding to the initiation region of its promoter (Rodriguez *et al.*, 2008). Boyd *et al.* (2013) confirmed the link between Tbx3 and E-cadherin expression and further demonstrated that knockdown of either *BRAF* or Tbx3 inhibited invasion by melanoma cells.

Cell migration and invasion is a complex multistep process requiring the detachment of cells from the matrix, cytoskeletal reorganization, reattachment, contraction, and matrix degradation. In melanoma, oncogenic *BRAF* is implicated in many of the key processes required for motility and invasion. Recent work has shown mutant *BRAF* to regulate directly the contractile ability of melanoma cells through the suppression of phosphodiesterase 5A, in turn leading to increased cGMP accumulation and the release of cytosolic calcium (Fedorenko *et al.*, 2011). At the same time, constitutive MAPK signaling directly contributes to remodeling of the actin cytoskeleton through the expression of RND3, a mediator in the cross talk between MAPK/ERK kinase signaling and the Rho/Rock/LIM kinase/Cofilin pathway (Fedorenko *et al.*, 2011). Taken together, it is likely that the reduced melanoma cell invasion observed by Boyd *et al.* (2013) following knockdown of either BRAF or Tbx3 is the result of the inhibition of the motile/invasive process at multiple levels. The link between *BRAF* mutation status and Tbx3 expression observed by Boyd *et al.* (2013) was also of clinical relevance, with a clear association being noted between *BRAF* mutational status and Tbx3 expression in human melanoma specimens. Interestingly, little correlation was seen between either *BRAF* mutational status or Tbx3 expression and E-cadherin

levels, suggesting that most melanomas lost E-cadherin expression and that multiple paths to E-cadherin deregulation exist.

We still do not fully understand all of the molecular steps required for oncogenic *BRAF* to transform melanocytes fully. Although the introduction of *BRAF* V600E into primary human melanocytes *in vitro* is associated with an initial burst of replication, these effects are short-lived and the cells eventually show signs of oncogene-induced senescence. A number of prior studies have suggested that Tbx3 (and the closely related Tbx2) promotes oncogenesis, partly through the suppression of senescence. Despite this, and the possibility that Tbx3 may limit the senescence response in melanocytes, Boyd *et al.* (2013) observed oncogenic *BRAF* to induce senescence even when Tbx3 expression was increased. These results add further weight to the emerging idea that activity in multiple signaling pathways may be required to fully drive melanocyte transformation. Although Boyd *et al.* (2013) suggested a role for $p16^{INK4A}$ in this process, other recent studies have implicated increased PI3K/AKT signaling, arising through either PTEN loss or increased AKT3 expression, in the escape of melanocytes from *BRAF*-mediated senescence (Vredeveld *et al.*, 2012). It is further likely that the PI3K/AKT signaling required for the escape from *BRAF*-mediated senescence may also enhance the EMT-like response of melanoma cells, leading to further potentiation of environmental escape (Kalluri and Weinberg, 2009). Insights into the potential cooperation between *BRAF* and the PI3K/AKT signaling pathways in driving the EMT-like response of melanoma cells are likely to prove useful in our understanding of the earliest stages of melanoma development.

The identification of mutant *BRAF* as a bona fide therapeutic target in 50% of melanoma patients and the subsequent clinical development of small-molecule BRAF inhibitors may revolutionize the treatment of disseminated melanoma (Fedorenko *et al.*, 2011). The findings of Boyd *et al.* (2013), as well as of others, showing that BRAF inhibition reverses partially the EMT-like state of melanoma, could represent a potential mechanism through which BRAF inhibitors such as vemurafenib and dabrafenib exert their effects. It is also intriguing that Tbx3, whose knockdown has multiple effects on melanoma cells, including the reduction of anchorage-independent growth and the abrogation of xenograft formation in immunocompromised mice, is suppressed by BRAF inhibitors (Peres *et al.*, 2010). We are only now beginning to understand how the acquisition of driver mutations, such as mutant *BRAF*, serve to rewire the signaling of melanocytes and drive them toward oncogenic transformation. These insights, linking mutant *BRAF* to E-cadherin and Tbx3, provide important clues about how melanoma may be managed therapeutically.

CONFLICT OF INTEREST

The author states no conflict of interest.

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See related article on pg 1286

MicroRNAs as an Emerging Target for Melanoma Therapy

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Despite the growing focus on microRNAs (miRNAs) as novel diagnostic tools and therapeutic targets in cancer, global characterization of miRNA expression patterns and their specific targets in melanoma has lagged. In this issue, Reuland *et al.* (2013) identify miR-26a as being specifically downregulated in human melanoma cells. They further establish *Silencer of Death Domains* as a novel target for miR-26a, which functionally mediates melanoma cell death. These findings suggest that miR-26a may serve as a promising novel therapy for subsets of melanoma.

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MicroRNAs (miRNAs) are small noncoding RNAs that regulate gene expression at the posttranscriptional level. Precursor miRNAs are initially transcribed in the nucleus and subsequently cleaved by RNAse III into mature miRNAs. Once in the cytoplasm, these molecules form an miRNA-induced silencing complex that binds to the 3′ untranslated regions of target transcripts.

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