
CONFLICT OF INTEREST
The authors state no conflict of interest.

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Get with the Program! Stemness and Reprogramming in Melanoma Metastasis

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Cancer cells are highly plastic and adopt multiple phenotypic states that contribute to tumor progression. Heppt et al. demonstrate that the homeodomain transcription factor Msx homeobox 1 reprograms melanoma cells to a precursor state associated with melanoma progression and increased liver metastasis. Identification of this new role for Msx homeobox 1 may facilitate the development of new therapies that limit melanoma dissemination.

Heppt et al. (2017) report new data that identify the homeodomain transcription factor Msx homeobox 1 (MSX1) as a master regulator that reprograms melanocytes to a de-differentiated, stem-like state (Figure 1). The authors further demonstrate that MSX1 plays an important role in melanoma progression, potentially through the regulation of liver metastasis development (Heppt et al., 2017). To date, MSX1 has been most widely studied in embryonic development, where it has been implicated in neural crest specification and primordial germ cell migration through the induction of

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multiple transcription factors including SNAIL, SLUG, and FOXD3 (Tribulo et al., 2003).

**Melanocyte Development and Tissue Homeostasis**

Under normal homeostasis, the behavior of nontransformed cells is subject to tight regulation by local host microenvironments. These interactions, which can be mediated through cell-cell adhesion, cell-matrix adhesion, gap junctions, and growth factors, give rise to critical signals that dictate whether cells grow, divide, or move (Smalley et al., 2005). Much of the control, and the tissue architecture of epithelial cell layers, is dependent on homotypic E-cadherin/E-cadherin-based adhesions between neighboring cells. This strong E-cadherin-mediated adhesion, along with that involving tight junction proteins, physically locks cells together ensuring tissue stability and optimal organ function. In early embryonic development, some epithelial cells downregulate their E-cadherin expression, acquire the phenotypic and motile characteristics of mesenchymal cells, and migrate to other anatomical sites. This process, called the epithelial-to-mesenchymal transition (EMT), is a key developmental program that permits cells to move over long distances before redifferentiating, restoring their epithelial characteristics, and re-establishing contact with their neighbors (a process called the mesenchymal-to-epithelial transition, or MET). In addition to being critical for normal organismal development, reactivation of the EMT transcriptional program can also occur in cancer, where it is frequently associated with metastasis (Smalley et al., 2005).

Melanocytes, the pigment-producing cells of the skin, develop from neural crest progenitor cells that have migrated to the skin and differentiated following the expression of the lineage-specific microphthalmia-associated transcription factor (MITF). Once located at the dermal-epidermal junction, differentiated melanocytes interact closely with the surrounding skin keratinocytes, in part through though E-cadherin-based cell-cell adhesion (Smalley et al., 2005). Keratinocytes regulate melanocytes, controlling everything from their growth to the synthesis and transport of melanin following ultraviolet light exposure (through the release of paracrine α-melanocyte stimulating hormone from the keratinocytes) (Kawakami and Fisher, 2017; Smalley et al., 2005). Escape of melanocytes from keratinocyte control is a key step in melanoma development, and it is absolutely required for nascent melanoma cells to both grow in an uncontrolled manner and migrate out of skin. This process is still incompletely understood, but it is frequently accompanied by an EMT-like switch associated with the loss of E-cadherin expression and acquisition of mesenchymal markers such as SNAIL, SLUG, TWIST, and ZEB1 (Caramel et al., 2013; Smalley et al., 2005) (Figure 1).

**The Link between Melanocyte Reprogramming and Melanoma Metastasis**

The goal of the study by Heppt et al. (2017) was to identify novel factors involved in melanocyte reprogramming and de-differentiation. This work is a continuation of a prior study in which the same authors identified the developmental regulator Notch-1 as a factor that reprogrammed fully-differentiated melanocytes into multipotent neural crest stem cells (Heppt et al., 2017). MSX1 was chosen based on its significantly increased expression in the Notch-1 reprogrammed cells. For this reason, and also because of its known role in neural crest differentiation, the authors hypothesized that MSX1 might drive melanocyte reprogramming and stemness.

The authors began by demonstrating that multiple, normal, nontransformed human melanocytes expressed very low levels of MSX1. Re-expression of MSX1 in the melanocytes led to

![Figure 1. MSX1 provides the link between melanocyte reprogramming and melanoma metastasis. Induction of MSX1 expression leads to decreased expression of melanocyte lineage factors and drives a switch to a neural crest-like phenotype. Increased expression of MSX1 in melanoma cells is associated with suppression of MITF expression and increased metastasis associated with increased p75 nerve growth factor receptor, Wnt5a, and ZEB1 expression. EMT, epithelial-to-mesenchymal transition; MITF, microphthalmia-associated transcription factor; MSX1, Msh homeobox 1.](https://www.jidonline.org)
their de-differentiation, an effect associated with depigmentation, altered morphology, and a decreased expression of melanocyte lineage markers (Heppt et al., 2017). Simultaneously, the MSX1 expressing melanocytes also showed decreased expression of E-cadherin, upregulated expression of the neural guidance/migration marker L1-CAM, and the neural crest marker p75 nerve growth factor receptor (CD271). In this neural crest-like state, the reprogrammed melanocytes showed characteristics of multipotency and could be differentiated into multiple other lineages including adipocytes and neuronal cells. In light of the obvious parallels between the MSX1-reprogrammed melanocyte state and the EMT and stemness common to melanoma, the authors next explored the role of MSX1 in melanoma progression by staining a series of melanoma specimens for MSX1 and interrogating the TCGA RNA-Seq dataset (Heppt et al., 2017). These analyses showed that MSX1 expression was significantly higher in melanoma metastases compared with primary melanomas from the earlier stage radial growth phase or vertical growth phases.

One key observation made by Heppt et al. related to the ability of MSX1 to inhibit expression of MITF, which itself is a master regulator of the melanocyte lineage (Kawakami and Fisher, 2017; Tribulo et al., 2003). The relationship between MITF expression and melanoma initiation and progression is a complex one (Kawakami and Fisher, 2017). Studies have shown that although amplified MITF functions as a melanoma oncogene in 5–20% of cases, some melanomas have low-to-absent MITF expression (Kawakami and Fisher, 2017). Expression levels of MITF also vary at the single cell level with MITF-high and MITF-low cells coexisting within the same tumor (Kawakami and Fisher, 2017). These MITF-high and MITF-low cells can readily interconvert, a phenomenon characterized as phenotype switching. This ability to switch between two different cellular states is thought to allow melanomas to adapt to different microenvironmental conditions. MITF expression levels also define distinct melanoma cell behaviors with MITF-high melanomas being more differentiated and proliferative and MITF-low melanomas being less pigmented, less differentiated, and highly invasive (Kawakami and Fisher, 2017). The identification of MSX1 as a regulator of MITF expression adds another layer of complexity to our understanding of phenotypic heterogeneity in melanoma and suggests that advanced melanomas share characteristics with cells of the primitive neural crest.

Perhaps the most provocative finding reported by Heppt et al. (2017) is the observation that knockdown of MSX1 reduced the seeding of melanoma cells to the liver after tail vein injection. As the liver is a common site of metastasis in melanoma, it is unclear whether this represents the suppression of an MSX1-specific liver metastasis program or instead reflects a general decrease in metastatic dissemination. To metastasize, cells must accomplish multiple tasks involving migration out of their local environment, resistance to anoikis, intravasation, survival in the circulation, extravasation, and the establishment of colonies at new organ sites (Smalley et al., 2005). From a mechanistic standpoint, it is easy to envisage how MSX1 expression promotes metastasis at multiple levels. Overexpression of MSX1 was associated with marked increases in melanocyte and melanoma cell invasion, an effect associated with increased expression of Wnt5A and ZEB1 (Richard et al., 2016) (Figure 1). Wnt5a is a known driver of invasion for both melanoma and nonmelanoma skin cancers, through its activation of protein kinase C and Rac1. Of these, Rac1 leads to the mesenchymal invasion of melanoma cells, which is characterized by actin polymerization secondary to the activation of PAK1 and WAVE and the secretion of proteases that “eat” holes through the extracellular matrix (Orgaz and Sanz-Moreno, 2013). It remains to be determined whether all of the cells in a metastasizing melanoma have high MSX1 expression or whether expression is more heterogeneous and focal, with only the most invasive cells at the leading edge having high MSX1 expression. There are certainly precedents for this. In nonmelanoma skin cancers, cells with very high Wnt5a expression are found mostly at invasive fronts. In melanoma, similar transitions occur at the invading edges of the tumors, with the cells frequently adopting a more aggressive, de-differentiated phenotype (Paraiso et al., 2015). The immunohistochemical staining for MSX1 provided by Heppt et al. (2017) seems to support this contention, with some of the strongest staining being observed in the invasive nests of melanoma cells within the dermis.

**Reprogramming and Drug Resistance?**

One issue not addressed by the current study is whether MSX1 expression plays a role in regulating drug sensitivity in melanoma. Previous work has shown that the adoption of an EMT, and a switch to a de-differentiated state, limits the response of melanoma cells to both serine/threonine-protein kinase B-raf (BRAF) inhibitors and immune checkpoint inhibitors (Hugo et al., 2016). Similar effects on sensitivity to BRAF and MAPK kinase inhibitors were also seen when direct MSX1 targets (such as ZEB1) were introduced into melanoma cells (Richard et al., 2016). In this instance, overexpression of ZEB1 also promoted a reversible phenotype switch leading to increased p75 nerve growth factor receptor expression and a reduction in MITF expression (Richard et al., 2016). Therapeutic targeting of ZEB1 restored the sensitivity of the cells to BRAF inhibition (Richard et al., 2016). Other studies have also confirmed that MITF-low melanoma cells are intrinsically resistant to MAPK pathway targeted drugs (Kawakami and Fisher, 2017). Another way that MSX1 could contribute to BRAF inhibitor resistance is through modulation of adaptive signaling. Studies performed in Xenopus have shown that MSX1 regulates the expression of FOXD3, a transcription factor implicated in stemness (Tribulo et al., 2003). In melanoma, FOXD3 is frequently induced after BRAF inhibitor treatment, and contributes to BRAF inhibitor tolerance through increased expression of the receptor tyrosine kinase ERBB3. It is therefore possible that MSX1 may directly influence the expression of receptor tyrosine kinases after BRAF inhibition, allowing for the recovery of adaptive prosurvival signaling pathways. The potential role for MSX1 in modulating BRAF inhibitor sensitivity at multiple levels makes it an attractive target for further study.
**Can We Prevent Phenotype Switching and Regulate Heterogeneity?**

The identification of MSX1 as a master regulator of the melanocyte lineage adds to our understanding of the role of cellular reprogramming in melanoma progression (Figure 1). To date, a wide variety of markers including MITF, JARID1B, ZEB1 (and now MSX1) have been described as markers of melanoma cell subpopulations with more primitive, stem-like behavior. Although the extent of overlap between all of these subgroups has not been elucidated, it seems likely that these phenotypic states are interrelated and there is already evidence for cross-regulation between MSX1, MITF, and ZEB1 (Heppt et al., 2017). The likelihood that these primitive cells are those that metastasize and maybe even resist therapies makes them attractive future targets for drug discovery. At this time we lack good strategies to target these multiple phenotypic states. The analysis of the melanoma TCGA dataset revealed only rare mutations in MSX1 (6 of 278 cases), suggesting that its regulation may be epigenetic (Heppt et al., 2017). There is already evidence that MITF expression can be regulated through histone deacetylase inhibitors and the anti-HIV drug nelfinavir (Kawakami and Fisher, 2017; Smith et al., 2016). Other recent studies have shown that the aggressive melanoma phenotypes emerging in BRAF inhibitor-treated patients can also be targeted and reverted through epigenetic inhibitors (Paraiso et al., 2015). Together these findings demonstrate that different melanoma cell states, and indeed phenotype switching, are therapeutically tractable. This is clearly an important area for future research. Continued progress in understanding the underlying biology of melanoma as well as melanocyte development will prove critical in the development of new combination therapies, allowing us to deliver more durable therapies to patients with advanced melanoma.

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**Opsin3—A Link to Visible Light-Induced Skin Pigmentation**

Subba Rao Gangi Setty

Skin pigmentation is primarily dependent on melanogenesis, a physiological process that occurs in melanosomes of melanocytes. Solar radiation modulates pigmentation through variety of signaling pathways, but the mechanism of visible light-induced hyperpigmentation remains uncharacterized. Passeron’s group recently reported that visible light stimulates opsin3-regulated calcium-dependent microphthalmia-associated transcription factor activation that increases pigment gene expression and that it also causes the clustering of melanogenic enzymes. Together, these processes possibly contribute to long-lasting hyperpigmentation in the melanoc ompetent skins.


Human skin pigmentation (scaled as Fitzpatrick phototype I–VI) depends on intra- and extracellular factors, including genotype, and is highly regulated and maintained via multiple signaling networks operating in melanocytes (Steingrimsson et al., 2004). Melanocytes populate the choroid of the eye, the inner ear, and hair follicles in addition to skin epidermis and produce melanin pigments in membrane-bound organelles called melanosomes. The amount of pigment that is produced in melanosomes and the extent of transfer of melanosomes to neighboring keratinocytes further determine skin color, which is modulated by solar radiation. These pigments absorb UV rays and protect the skin from ionizing radiation. Solar radiation...