head region to changes in nucleotide concentrations.

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Midkine and Melanoma Metastasis: A Malevolent Mix

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Using an *in vivo* reporter for lymphangiogenesis, a recent study in *Nature* from Olmeda et al. (2017) describes a new subset of melanomas that induce systemic pre-conditioning of distant organs for formation of tumor metastatic niches, and identifies the responsible factor as the pleiotropic cytokine midkine.

Melanoma is the most serious type of skin cancer because of its early spread via the lymphatic vessels into lymph nodes and distant organs. Luckily, recent breakthroughs in the Braf and MEK inhibitor and immune checkpoint inhibition therapies have impressively improved prognosis in melanoma (Luke et al., 2017), increasing the long-term survival in the majority of patients. Despite this, metastasis of melanoma is still a significant issue, and, although melanoma patients die of metastatic disease, a detailed mechanistic understanding of how this metastasis occurs has been lacking. In particular, the timing and routes of tumor cell dissemination, as well as dormancy versus growth in the metastatic sites, are unclear. In contrast to, e.g., colorectal carcinomas (Naxerova et al., 2017), the favored route of melanoma metastasis to distant organs is via the lymphatic vasculature of the skin, but the main sites of entry of the melanoma cells into the blood stream are not known. After lymphatic invasion, entry into blood circulation might happen in the lymph nodes, or perhaps the metastatic cells need to first traverse through colonization of secondary lymph nodes to reach blood circulation (Figure 1).

Signaling by the major lymphangiogenic factors VEGF-C and VEGF-D via their cognate receptor VEGFR3 promotes lymphatic metastasis in mouse models, and both VEGF-C and lymphangiogenesis correlate with lymphatic metastasis in human cancers (Karaman and Detmar, 2014). Although the first inhibitor of lymphangiogenesis and lymphatic metastasis was identified in 2001 (Karpanen et al., 2001), inhibition of such modes of metastasis has been an elusive goal for the pharmaceutical industry. Perhaps one reason for this failure is the idea that primary tumors could facilitate metastasis by pre-conditioning distant sites for metastasis arrival, a theory that has been supported by recent evidence (Peinado et al., 2017). In a striking recent report published by Olmeda et al. (2017) in *Nature*, the authors discover a new pre-conditioning factor in melanoma metastasis. The authors used live imaging of a genetic VEGFR3-luciferase reporter to detect lymphangiogenesis and thus distal pre-metastatic niches (Martínez-Corral et al., 2012). This technique was used for human melanoma cell lines and patient-derived xenografts, in addition to state-of-the art genetic melanoma mouse models. This allowed the authors to identify melanomas in which neo-lymphangiogenesis at distal sites was found prior to or in the absence of noticeable lymphangiogenesis at the site of the primary tumor. By surgically removing the cutaneous lesions and analyzing these distal lymphangiogenic organ sites, the authors found that they were potential pre-metastatic niches. The authors then performed a proteomic analysis of the exosome cargo from the corresponding human metastatic melanoma cell lines for factors that could pre-condition these distal pre-metastatic niches.

The top candidate mediator of melanoma lymphangiogenesis and metastasis was found to be the heparan sulfate binding factor midkine (MDK). The authors found that an overwhelming majority of MDK produced by melanoma cells was in a soluble, secreted form. Silencing of MDK decreased lymphangiogenesis and metastasis into lymph nodes and lungs, while MDK overexpression increased these in immunodeficient *nu/nu* mice. Interestingly, MDK was found to accumulate in association with lymphatic vessels in lungs and liver before tumor colonization and to promote mTOR activation, VEGFR3 expression, and cell proliferation in cultures of human lymphatic endothelial

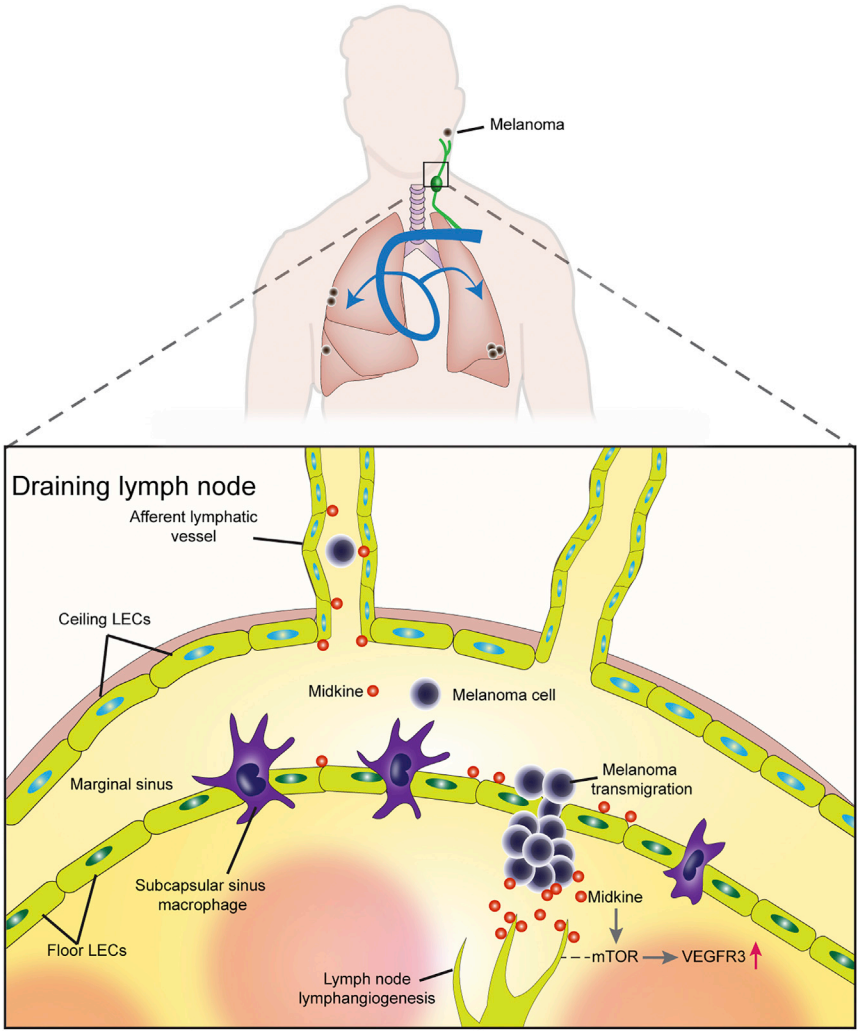

Figure 1. Midkine Expression by Melanomas Promotes Lymphovascular Niche Formation and Tumor Metastasis

Olmeda et al. (2017) show that midkine (MDK) expression increases during the progression of human melanomas. In a subset of human melanomas, MDK is produced at high levels, and in mice, tumor xenografts of these cancers stimulate systemic lymphangiogenesis and tumor cell transmigration through the lymphatic endothelium in pre-metastatic sites, such as the draining lymph nodes. MDK binds heparan sulfate and lymphatic endothelial cells (LECs), in which it activates mTOR signaling to increase the expression of VEGFR3, through which major lymphangiogenic signals are transduced.

cells, providing a possible mechanism for its action in vivo via the mTOR-VEGF-C-VEGFR3 pathway (Figure 1). Another mechanism of action that was associated with MDK expression was enhanced transmigration of melanoma cells through lymphatic endothelium *in vitro* and into lymph nodes (Figure 1).

Retrospective immunohistochemical analysis by Olmeda et al. (2017) also indicated that over half of the human melanomas examined express more MDK than benign moles and that a striking upregulation of MDK expression occurs in local, lymph node, and distant metastases. Furthermore, stage II or III patients with high expression of MDK in sentinel lymph nodes had shorter disease-free survival times than those with low expression.

The effects of MDK on cells and its receptors are numerous (Sorrelle et al., 2017), raising the question of which of its reported proximal signal transduction mechanisms are at play in melanoma metastasis. Furthermore, several other questions are raised by the results presented by Olmeda et al. (2017).

If MDK is expressed in the tumor cells and acts via the mTOR-VEGFR3 pathway in LECs to upregulate VEGFR3, why does it stimulate VEGFR3-Luc activity and lymphangiogenesis preferentially in distant organs rather than in the primary tumor? How can MDK promote intravasation into lymphatic vessels if it acts primarily in the (pre)metastatic sites? Because MDK expression is also increased in multiple other types of cancer and because it provides a prognostic biomarker in some of these, does it enhance metastasis to lymph nodes and distant organs in non-melanoma cancers?

Is the effect of MDK dependent on parallel stimulation of lymphangiogenesis by the VEGF-C/VEGFR3 pathway? Although analysis of VEGF-C production in cultured melanoma cells indicated that this is perhaps not the case, one should remember that tumors derived from cell lines that do not constitutively express VEGF-C or VEGF-D in tissue culture can nevertheless express one or both of these factors *in vivo* (Krishnan et al., 2003). Both tumor and stromal cells contribute to this expression, suggesting that tumor cell-host interactions determine tumor expression of VEGF-C and VEGF-D. MDK also has strong immunoregulatory functions (Sorrelle et al., 2017), and stromal inflammatory cells in the lymph nodes and lungs could be major effectors of MDK action. Furthermore, RNA expression is just a prerequisite for activity, because these lymphangiogenic factors are activated by a proteolytic mechanism that requires at least two other factors (Jeltsch et al., 2014).

How lymphangiogenesis at sites of distant metastasis contributes to tumor progression is not clear. Perhaps it is the lymphatic endothelial transmigration of tumor cells that is most important. Rapidly developing methods for imaging of whole organs and mice by applying tissue-clearing methods should soon allow such questions to be answered at single-cell resolution. The translational value of the findings of Olmeda et al. (2017) will undoubtedly be further validated in their transgenic melanoma model crossed to MDK-deficient background and in experiments testing the inhibition of melanoma metastasis by using MDK blocking antibodies.

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Appetite for Neurogenesis

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Adult neural stem cells (NSCs) in the ventricular-subventricular zone (V-SVZ) produce diverse olfactory bulb (OB) neurons. In a recent paper in *Science*, Paul et al. (2017) show that hypothalamic propiomelanocortin (POMC) neurons innervate the anterior ventral V-SVZ and regulate deep granule interneuron production depending on feeding behavior.

New olfactory neurons are produced throughout life from NSCs in the adult murine V-SVZ (Bjornsson et al., 2015). The NSCs (type B cells) are GFAP$^+$ astrocyte-like cells that exist in either a quiescent (qNSCs) or an activated (aNSCs) state. qNSCs are Nestin-negative, divide slowly, and rarely produce clones in vitro. Conversely, aNSCs are Nestin$^+$, divide rapidly, and readily give rise to clones. aNSCs produce transit-amplifying type C cells, which in turn produce doublecortin$^+$ type A neuroblasts that proliferate and migrate through the rostral migratory stream to the OB. Tract-tracing and fate-mapping studies have shown that NSCs in different domains of the V-SVZ express specific transcription factors and specify diverse OB interneuron populations (Figure 1). Prior studies demonstrated that the Nkx6.2$^+$ population located in the anterior ventral V-SVZ is stimulated by Sonic hedgehog, which balances aNSCs and qNSCs (Daynac et al., 2016; Merkle et al., 2014). How other NSC subtypes are regulated is an active area of study.

The NSC niche serves as a rich source of external regulatory cues via blood vessels, ependymal cells, microglia, extracellular matrix, nerve fibers, and cerebral spinal fluid (CSF) (Bjornsson et al., 2015). Diverse neuronal inputs can also regulate adult neurogenesis via direct innervation of the V-SVZ by dopamine (Lennington et al., 2011), serotonin, and choline acetyltransferase neurons (Bjornsson et al., 2015). In a recent issue of *Science*, Paul et al. (2017) advance the field by demonstrating that POMC neurons releasing β endorphin directly contact and selectively regulate a subset of regionally located V-SVZ NSCs.

Paul et al. (2017) used fluorescence-activated cell sorting to purify V-SVZ qNSCs and screened for compounds that would transition these to aNSCs. Excitingly, both ICI 204448, an agonist of the kappa opioid receptor, and the endogenous ligand β endorphin were found to activate previously qNSCs *in vitro*, stimulating them to proliferate and produce Nestin$^+$ progeny.

Next, the authors demonstrated that *in vivo* intraventricular administration of β endorphin receptor agonists and antagonists regulated proliferation of a distinct population of NSCs, the Nkx2.1$^+$ cells located in the anterior ventral SVZ. Moreover, they showed that these Nkx2.1$^+$ V-SVZ cells express the β endorphin kappa opioid receptor (Oprk1), whereas dorsal V-SVZ cells did not, illustrating how NSCs can be regionally specified to respond to a particular neuronal input.

The β endorphin neuropeptide is produced by POMC neurons in the pituitary and the arcuate nucleus (ARC) of the hypothalamus (Figures 1A and 1B). Paul et al. (2017) discovered that the Nkx2.1$^+$ NSCs and cells in their niche are directly innervated by ARC POMC neurons. Further, activation of these POMC neurons increased proliferation of Nkx2.1$^+$ NSCs, whereas their ablation decreased proliferation of Nkx2.1$^+$ NSCs and decreased the numbers of their progeny, the deep granule cell interneurons in the OB. To determine whether these treatments can activate previously qNSCs *in vivo*, it would be worthwhile to test whether β endorphins are still effective