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Lymphangiogenesis: From passive disseminator to dynamic metastatic enabler

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The lymphatic vasculature drains fluid and cells from tissues and channels them to the lymph nodes, where they encounter immune cells, constituting a critical component of immune function and tissue fluid homeostasis. It has been hypothesized that the formation of new lymphatics (lymphangiogenesis) in tumors can facilitate cancer cell transport to the lymph node, whose permissive microenvironment would favor their survival before disseminating to other organs. However, lymphatic vessels found inside tumors are generally not functional and surgical resection of sentinel lymph nodes does not improve melanoma patient's outcome (Morton et al., 2014), questioning the direct involvement of lymphangiogenesis in metastasis. The recent study published by Olmeda and colleagues in *Nature* provides evidence of the functional relevance of lymphangiogenesis in melanoma colonization to distant organs, and in so doing transforms the notion of a passive pipeline into a dynamic force in the metastatic process. Furthermore, the authors employ sophisticated genetically engineered mouse (GEM) models to monitor systemic lymphatic formation, offering a promising tool for the early detection and mechanistic study of metastasis.

Lymphangiogenesis comprises several steps, including migration, proliferation, and sprouting of lymphatic endothelial cells (LEC), which are triggered by stimulation of vascular endothelial growth factor receptor 3 (VEGFR3) by VEGFC or D, resulting in the formation of lymphatics. Olmeda et al. employed a dual GFP and luciferase reporter cassette driven by the endogenous Vegfr3 promoter to visualize the systemic induction of lymphangiogenesis in several melanoma mouse models in real time. This Vegfr3 reporter mouse was shown to be a useful platform to study the roles of lymphangiogenesis in wound healing, inflammation, and tumor progression (Martinez-Corral et al., 2012). Four patterns of lymphangiogenesis induction by human melanoma cells implanted into immunocompromised mice were identified by Olmeda et al., characterized by Vegfr3-driven luciferase expression in either primary tumors, distal/lymph node metastases, both, or neither. Surprisingly, the metastatic capability of melanoma correlated with systemic lymphangiogenesis (i.e., occurring in lymph nodes and distal organs), but was independent of the induction at the primary tumor site. Future studies might elucidate whether this pattern of dissemination is shared by other tumor types or is specific for melanoma. Interestingly, the systemic induction of lymphangiogenesis preceded the colonization of lymph nodes and lungs by melanoma cells, consistent with a role for lymphangiogenesis in the creation of a premetastatic niche. Moreover, resection of primary tumors generated by transplantation of melanoma cell lines or patient-derived xenografts (PDXs) promoted a significant decrease in Vegfr3-driven luciferase expression, suggesting that tumor cells at primary sites are the main source of the factors that distally induce lymphangiogenesis. The

recovery of luciferase signal in this model was also observed prior to melanoma relapse and metastatic growth in the lymph nodes and lungs. These findings raise the possibility that circulating tumor cells could serve as additional inducers of lymphangiogenesis, opening new areas for future studies.

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An analysis of the melanoma secretome (including exosome-associated and soluble factors) led the authors to identify the heparin-binding factor midkine (MDK) as a major player in this process. MDK was secreted by highly metastatic melanoma cells, and accumulated in active lymphangiogenesis areas and in the tip cells of sprouting lymphatics at the metastatic sites, such as lymph nodes and lungs. Olmeda and colleagues found significant activation of the mTOR pathway by secreted MDK in LEC concomitant with upregulation of VEGFR3 expression, which stimulated their sprouting and proliferation. Using a variety of in vitro and in vivo assays, including time-lapse microscopy of melanoma cells and LEC cocultures, transmigration assays, histological immunostaining, and intravital imaging of lymph node metastases, the authors also showed that MDK enhanced adhesion and transmigration of melanoma cells through LEC. Loss- and gain- of- function studies demonstrated that MDK confers upon melanoma cells the ability to systemically induce lymphangiogenesis and metastasize to lymph nodes and lungs. Previous studies had linked MDK to the activation of angiogenesis driven by hypoxia in normal and tumoral tissues (Weckbach et al., 2012). Conversely, Olmeda et al. did not find any obvious effect of MDK knockdown on the blood vasculature or endothelial cell proliferation. Whether MDK is also essential for cancer metastasis via angiogenesis in other cellular contexts could be addressed in the future. It will also be critical to identify which factors determine whether lymphatics or blood vessels will be the route for dissemination.

The study by Olmeda et al. has provided significant results with direct applicability to clinical practice. Importantly, high MDK expression in lymph nodes was correlated with worse disease-free survival of melanoma patients. The prognostic value of MDK was independent of age, gender, or Breslow depth and included melanoma-negative sentinel lymph nodes. Therefore, MDK expression in lymph nodes could be a useful biomarker for predicting metastatic disease. Overall, the findings in this study offer MDK as an attractive target for the treatment of melanoma at the premetastatic stage. As MDK is a secreted factor, the development of blocking antibodies could be a feasible strategy. Alternatively, the blockade of lymphangiogenesis could be explored as an adjuvant approach to melanoma treatment. Currently, there are no approved molecules that specifically target lymphangiogenesis. However, preclinical studies have shown impaired lymphangiogenesis in head and neck cancer and breast cancer upon treatment with mTOR and VEGFR3 inhibitors, respectively, associated with a significant decrease in metastasis (Garcia-Caballero et al., 2017; Patel et al., 2011).

Removal of sentinel lymph nodes is a common practice for the diagnosis and prevention of metastatic disease in melanoma. However, it is still not clear whether the benefit for melanoma patients justifies the side effects associated with this surgery (Morton et al., 2014). Transplantation of melanoma PDXs into the GEM reporter model described by

Olmeda and colleagues could serve as avatars to characterize the pattern of lymphangiogenesis induction exhibited by individual patient melanomas, and to determine whether a correlation with metastasis risk truly exists. Could the role of lymphatic vasculature as a dynamic enabler of melanoma metastasis be exploited as a promising diagnostic tool? Undoubtedly, the work of Olmeda et al. has laid a strong basis for future translational studies in this direction.

REFERENCES

- Garcia-Caballero M, Paupert J, Blacher S, Van de Velde M, Quesada AR, Medina MA, & Noel A (2017). Targeting VEGFR-3/-2 signaling pathways with AD0157: A potential strategy against tumor-associated lymphangiogenesis and lymphatic metastases. *Journal of Hematology & Oncology*, 10, 122. [PubMed: 28629427]
- Martinez-Corral I, Olmeda D, Dieguez-Hurtado R, Tammela T, Alitalo K, & Ortega S (2012). In vivo imaging of lymphatic vessels in development, wound healing, inflammation, and tumor metastasis. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 6223–6228. [PubMed: 22474390]
- Morton DL, Thompson JF, Cochran AJ, Mozzillo N, Nieweg OE, Roses DF, ... Faries MB (2014). Final trial report of sentinel-node biopsy versus nodal observation in melanoma. *New England Journal of Medicine*, 370, 599–609. [PubMed: 24521106]
- Patel V, Marsh CA, Dorsam RT, Mikelis CM, Masedunskas A, Amornphimoltham P, ... Gutkind JS (2011). Decreased lymphangiogenesis and lymph node metastasis by mTOR inhibition in head and neck cancer. *Cancer Research*, 71, 7103–7112. [PubMed: 21975930]
- Weckbach LT, Groesser L, Borgolte J, Pagel JJ, Pogoda F, Schymeinsky J, ... Walzog B (2012). Midkine acts as proangiogenic cytokine in hypoxia-induced angiogenesis. *American Journal of Physiology. Heart and Circulatory Physiology*, 303, H429–H438. [PubMed: 22707563]