VEGF-D(ilated) Lymphatics as Gateways to Metastasis

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DOI 10.1016/j.ccr.2012.01.012

VEGF-C and VEGF-D have been implicated in lymphatic metastasis, mainly as inducers of new intra/peritumoral capillary lymphatics. In this issue of *Cancer Cell*, Karnezis and colleagues challenge this notion and demonstrate that tumor-derived VEGF-D promotes metastasis by causing prostaglandin-dependent dilation of collecting lymphatics outside of the tumor mass.

Metastasis defines the progressive, systemic, and intractable nature of late-stage human cancers. Implicitly, this process involves trafficking of cancer cells within blood and lymphatic compartments to sites of their secondary growth in distant organs and regional lymph nodes, respectively (Fidler, 2003). By extension, the corresponding processes of tumor-induced formation of new blood vessels (angiogenesis) and lymphatics (lymphangiogenesis) are often viewed as prerequisites for the onset of hematogenous and lymphatic dissemination. Either of the resulting microvascular networks may constitute a point of entry for cancer cells into the vascular system and a rate limiting step in disease spreading (Folkman, 2007; Alitalo, 2011). In spite of some controversy as to the causal role of lymph node metastases as "launch pads" of systemic dissemination (Sleeman and Thiele, 2009), the inherent appeal of this possibility has contributed to a growing interest in targeting lymphangiogenesis as an early pro-metastatic switch in cancer (Alitalo, 2011).

Much of the effort in this area has centered around obliteration of major lymphangiogenic growth factor pathways triggered by members of the vascular endothelial growth factor (VEGF) family, especially VEGF-C, VEGF-D, and to some extent VEGF-A, all of which may be expressed by metastatic cancer cells. The respective receptors for these factors, VEGFR3 and VEGFR2, are present on lymphatic endothelial cells (LECs), which can be distinguished from their vascular counterparts by patterns of gene expression and distinct molecular markers (LYVE-1, Prox1, and podoplanin) (Alitalo, 2011). LECs line both the terminal, thin walled lymphatics, devoid of supporting mural cells, and their draining contractile ducts, collecting lymphatic vessels (CLVs), which contain mural cells and pass the interstitial fluid (and cancer cells) to the regional lymph nodes.

Several specific monoclonal antibodies, soluble receptors, and small molecule inhibitors have been developed to block VEGF/VEGF receptor (VEGFR) pathways and impede lymphangiogenesis. In addition, the expression of VEGFs by cancer cells can be inhibited using non-steroidal anti-inflammatory drugs (NSAIDs) and other agents. The intuitively obvious way to observe whether these drugs actually work in cancer would be to follow the expected decline in density of capillary lymphatics within, and adjacent to, the tumor mass, using LEC markers such as LYVE-1. Indeed, such effects have been observed and may, at least in some cases, be rate-limiting for lymphatic and systemic metastasis.

As it turns out, clues as to the role of VEGFs in lymph node metastasis may also be found outside of the proverbial "box," i.e., away from the tumor masses, and within their draining CLVs. A compelling example of this scenario is described by Karnezis et al., (2012) in this issue of *Cancer Cell*. These authors set out to explore processes by which tumors expressing VEGF-D trigger lymph node metastasis. Although such tumors do contain rich networks of capillary lymphatics, these investigators noticed that what separated them from their non-metastatic counterparts was a startling (macroscopic) enlargement of CLVs draining the tumor basin to sentinel lymph nodes. The striking images of this CLV dilation suggest that the lymphatic influence of a growing cancer is not confined to its physical boundaries, but extends far beyond. What might be the mechanism by which VEGF-D-expressing tumor cells exert such a long distance influence (over tens of millimeters), and what are the consequences?

In search for answers Karnezis et al., (2012) first documented that CVL dilation is, indeed, directly VEGF-D-dependent. For example, this effect was absent in the case of non-metastic and VEGF-D non-expressing tumors or when VEGF-D was replaced with VEGF-A. Likewise, treatment with neutralizing antibodies against VEGF-D (VD1), VEGFR3 (mF4-31C1), or VEGFR2 (DC101) abolished CLV dilation. These observations suggest that VEGF-D causes dilation through cooperative activation of VEGFR3 and VEGFR2 in LECs that are located in the extra-tumoral CLV segments. To determine the nature of these responses, CLVs were isolated, and their LEC populations were purified and profiled for gene expression. This revealed a distinct molecular signature of these cells, including their ability to markedly downregulate prostaglandin dehydrogenase (*PGDH*) in the presence of VEGF-D. PGDH breaks down prostaglandins (e.g., PGE2), thereby opposing the action of the prostaglandin synthesis pathway driven by cyclooxygenase 2. Thus, downregulation of PGDH in LECs exposed to VEGF-D raises the levels of circulating prostaglandins, which in turn act on mural cells within tumor-related CLVs, causing their dilation.

These events have profound consequences for metastasis. Karnezis et al., (2012) demonstrated that essentially all treatments that counteracted CLV dilation also diminished the metastatic load in draining lymph nodes. Interestingly, Etodolac, an NSAID, triggers these effects essentially without changing the lymphatic or blood vessel density within

the primary tumor, which suggests that CLV dilation may have a far more central role in the metastatic process than hitherto appreciated. Interestingly, Etodolac also diminished metastatic burden in the lung. These results suggest that a level of control over the lymphatic and systemic dissemination could potentially be achieved by administration of relatively safe anti-inflammatory agents.

This provocative study adds an important dimension to the process that might be viewed as vascular system "conditioning" for cancer metastasis. While the focus of the present study is on CLV dilation, others observed lymphangiogenesis within lymph nodes prior to their metastatic colonization (Tobler and Detmar, 2006), a process that may be attributed to remote influences of growth factors or exosomes (Hood et al., 2011). Analogous pre-metastatic niches were also described at sites of blood borne metastases (Kaplan et al., 2005).

The enlargement of macroscopic vessels located outside of a growing tumor is not restricted to CLVs. Similar increases in diameter are often observed in the case of blood vessels that supply tumor microcirculation (feeding arteries and collecting veins), which is also apparent from some of the images included in the study by Karnezis et al., (2012). Although this is a commonly observed phenomenon, the underlying biological process has thus far attracted minimal attention (Yu and Rak, 2003). In contrast to angiogenesis, which occurs at the level of microscopic capillaries (Carmeliet and Jain, 2011), formation of larger tumor-feeding blood vessels may involve such mechanisms as dilation, similar to that occurring in CLVs, or circumferential growth ("tumor arteriogenesis") (Yu and Rak, 2003). Whether such macroscopic changes control tumor microenvironment, growth, or hematogenous metastasis (by analogy to CLVs) remains to be studied.

The novel and fascinating link between CLV dilation and lymphatic metastasis described by these authors raises several important questions. For example, how does CLV dilation promote metastasis? Is this merely a wider conduit ("plumbing") effect, or does it involve more subtle regulatory mechanisms (e.g., tumor-LEC interactions)? Since the VEGF-D-induced increase in prostaglandin levels is detected in peripheral blood, could such a change be indicative of impending lymphatic metastasis in the clinic? How early in progression of human cancers would increase in prostaglandins occur, and how discrete, how detectable, would this event be? What systemic consequences may be associated with VEGF-D-induced increase in prostaglandins in blood, e.g., for the vascular system? What turns on lymphangiogenic growth factors in metastatic cancers, and is there a link between oncogenic pathways and CLV dilation?

It is fascinating to think that a pharmacological blockade of the pathological CLV dilation and metastasis could be achieved with already available agents (VEGF/VEGFR3/2 inhibitors and NSAIDs). However, one wonders whether such treatment could interfere with the lymph outflow from the primary tumor mass leading to a build up of interstitial fluid pressure (IFP)? Increase in IFP has been linked to impaired drug delivery and could result in vascular compression, hypoxia, and perhaps in hematogenous metastasis. It is unclear if any of these effects might accompany therapeutic interference with CLV dilation. Indeed, the work of Karnezis et al., (2012) opens up several new lines of inquiry and a new domain in the field of lymphangiogenesis and cancer progression.

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aSIRTing Control over Cancer Stem Cells

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DOI 10.1016/j.ccr.2012.01.014

Cancer stem cells lie at the root of chronic myelogenous leukemia (CML) and mediate its continued growth. Their resistance to current therapies results in an inability to eradicate the disease. In this issue of *Cancer Cell*, Li et al. identify SIRT1 as a new target for eliminating CML cancer stem cells.

Chronic myelogenous leukemia (CML) is a cancer that begins in hematopoietic stem cells. Triggered by the BCR-ABL translocation (Melo and Barnes, 2007), additional mutations can induce its progression from a slow-growing chronic