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Role of lymphatic vasculature in regional and distant metastases

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ABSTRACT

In cancer, lymphatic vasculature has been traditionally viewed only as a transportation system for metastatic cells. It has now become clear that lymphatics perform many additional functions which could influence cancer progression. Lymphangiogenesis, induced at the primary tumor site and at distant sites, potently augments metastasis. Lymphatic endothelial cells (LECs) control tumor cell entry and exit from the lymphatic vessels. LECs also control immune cell traffic and directly modulate adaptive immune responses. This review highlights advances in our understanding of the mechanisms by which lymphatic vessels, and in particular lymphatic endothelium, impact metastasis.

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Introduction

Metastasis is the main cause of treatment failure and death for cancer patients. The involvement of lymphatic system with cancer has long been recognized as an important indicator of cancer aggressiveness. Lymph node status is one of the key parameters used for determining the stage of disease progression and it is a powerful predictor of patient survival (Edge, 2010). Patients with lymph node metastases are also more likely to present with disease recurrence (Rosen, 2008). However, the causal link between lymphatic dissemination and the negative outcome is not understood and how exactly the lymphatic system contributes to cancer progression from localized to systemic, disseminated disease remains a critical open question. Although the number of publications on the topic of cancer lymphatics has been growing steadily over the past decade, there is still a lot to be learned. This review highlights advances in our understanding of the mechanisms by which lymphatic vessels, and in particular lymphatic endothelium, impact metastasis.

Tumor lymphangiogenesis

Upon identification of VEGF-C and VEGF-D as lymphangiogenesis factors (Jeltsch et al., 1997; Joukov et al., 1996; Joukov et al., 1997), we and others have reported more than a decade ago that induction

Abbreviations: CCL, CC chemokine ligand; CCR, CC chemokine receptor; CXCR, CXC chemokine receptor; DC, dendritic cell; IL, interleukin; LEC, lymphatic endothelial cells; LN, lymph node; MR, mannose receptor; SCS, subcapsular sinus; SEM, scanning electron microscopy; TNF-α, tumor necrosis factor alpha.

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of lymphangiogenesis by the tumor facilitates metastatic spread (Mandriota et al., 2001; Skobe et al., 2001; Stacker et al., 2001). Since then, work from many laboratories has recapitulated these findings in numerous animal models and further showed that inhibition of lymphangiogenesis by blockade of VEGF-C or its receptor VEGFR-3, prevents lymph node metastases without significantly affecting primary tumor growth (Brakenhielm et al., 2007; Burton et al., 2008; Chen et al., 2005; He et al., 2005; Kawakami et al., 2005; Krishnan et al., 2003; Lin et al., 2005; Mandriota et al., 2001; Mattila et al., 2002; Skobe et al., 2001; Yanai et al., 2001). VEGF-C also facilitates metastatic spread to distant sites and, conversely, blocking VEGF-C or VEGFR-3 inhibits distant metastases in majority of experimental models (Brakenhielm et al., 2007; Burton et al., 2008; Chen et al., 2005; Krishnan et al., 2003; Lin et al., 2005; Roberts et al., 2006). In agreement with the preclinical data, numerous clinical studies reaffirmed the negative correlation between VEGF-C, lymphangiogenesis and patient outcome (Alitalo and Carmeliet, 2002; Ding et al., 2007; Furudoi et al., 2002; Miyazaki et al., 2008; Mohammed et al., 2007; Pepper et al., 2003; Swartz and Skobe, 2001; Tsutsumi et al., 2005). VEGF-C and VEGF-D are most specific and best studied lymphangiogenesis factors, however, tumor lymphangiogenesis can be mediated also by several pleiotropic factors, including PDGF-BB, IGFs, FGF2, HGF, Ang2, adrenomedulin and IL-7 (Zheng et al., 2014).

Lymphangiogenesis associated with the primary tumor is thought to increase metastasis by increasing the probability for tumor cells to enter into the lymphatic vessels. Large numbers of newly generated lymphatics create more opportunities for tumor cell exit and close proximity of tumor cells to LECs could make more tumor cells respond to LEC-derived chemokines and be mobilized into the lymphatics. Furthermore, gene-profiling data of tumor-activated and quiescent lymphatic endothelium showed significantly different expression profile, suggesting that tumor cells may interact differently with the pre-existing and with the newly formed lymphatics (Clasper et al., 2008). The nature and significance of that cross-talk, however, remain to be elucidated. Importantly, while tumor lymphangiogenesis profoundly increases metastatic spread, it is not an obligatory step for metastasis. Controversy on this topic stems from the assumption that if angiogenesis is required for tumor growth, by inference, lymphangiogenesis must be a requirement for metastasis. However, paradigms established for tumor angiogenesis cannot be extrapolated on lymphangiogenesis, since function of lymphatics and blood vessels in tumors is very different despite the fact that the endothelial biology of these two vascular systems is shared on many levels.

Interestingly, lymphangiogenesis in the sentinel lymph nodes has been shown to precede lymph node metastasis in several studies (Dadras et al., 2005; Harrell et al., 2007; Hirakawa et al., 2007; Hirakawa et al., 2005; Ruddell et al., 2008; Van den Eynden et al., 2006; Van den Eynden et al., 2007). Lymph node lymphangiogenesis is a component of the normal host immune response (Angeli et al., 2006; Kim et al., 2012; Randolph et al., 2005), which in the tumor setting is thought to enhance metastasis by creating a pre-metastatic niche. Because selective inhibition of lymph node lymphangiogenesis is difficult to achieve, this concept is derived mainly from correlative studies and more work is needed to elucidate exact role of LN lymphangiogenesis in cancer spread. Lymphangiogenesis has also been documented within metastases in the sentinel and more distal lymph nodes (Kerjaschki et al., 2011). Furthermore, this study indicated that tumor cell invasion into the newly formed lymphatic vessels in LN metastases and formation of tumor emboli is necessary for tumor dissemination into more distal lymph nodes (Kerjaschki et al., 2011).

Mechanisms of lymph node metastasis

Many important questions about lymph node metastasis remain unresolved to date. Lymph nodes are usually first sites of detectable metastases, which could be due to the preference of tumor cells to enter into the lymphatic vessels. It is not known however, whether such preference exists and whether tumor cell rate of entry into the lymphatic and blood vessels is different. Alternatively, early metastasis in the lymph nodes could be a result of survival or growth advantage within the lymph node microenvironment. Another key unresolved question is to which extent lymph node metastases directly contribute to the formation of distant metastases. While these issues have been frequently debated, there is no data to clearly support or oppose any of the aforementioned concepts.

Over decades, lymphatics were portrayed as passive participants in metastasis and regarded mainly as a transportation system. Recent studies, however, indicate that tumor cells are guided into the lymphatic vessels by chemokines produced by lymphatic endothelium (Ben-Baruch, 2008; Das and Skobe, 2008). CCL21 is constitutively expressed by the lymphatic vessels (Gunn et al., 1998; Kerjaschki et al., 2004; Podgrabinska et al., 2002; Shields et al., 2007), immobilized by binding to heparin sulfates and forms steep gradients within the perilymphatic interstitium (Haessler et al., 2011; Schumann et al., 2010; Weber et al., 2013). These gradients induce directed migration of dendritic cells towards lymphatics from a distance of up to 90 μm (Weber et al., 2013), suggesting that melanoma and breast cancer cells expressing CCR7 receptor (Houshmand and Zlotnik, 2003; Muller et al., 2001) could also be guided into the vessels by such haptotactic chemokine gradients. Overexpression of CCR7 in melanoma has indeed been shown to facilitate lymph node metastasis in a mouse model (Wiley et al., 2001) and clinical studies have confirmed the correlation between CCR7 expression and lymph node metastasis (Cabioglu et al., 2005; Ishigami et al., 2007; Mashino et al., 2002). CXCL12 is another chemokine that has been shown to facilitate lymph node metastasis of CXCR4+ tumor cells (Hirakawa et al., 2009; Muller et al., 2001; Uchida et al., 2007). CXCL12 is upregulated on lymphatic vessels in the primary tumor and it has been shown to promote recruitment of CXCR4+/CD133+ melanoma cells into the proximity of lymphatic endothelium. However, direct evidence for its role in directing cells into the lymphatic capillaries is lacking. Several studies suggested that macrophage mannose receptor I (MR) and CLEVER-1 may be important mediators of cancer cell adhesion to lymphatic endothelium (Irjala et al., 2003; Irjala et al., 2001). MR and CLEVER-1 expression has been detected on tumor lymphatic vessels and it was associated with increased lymph node metastases (Irjala et al., 2003). There is no evidence, however, that adhesive interactions with LECs are indeed required for tumor cell entry into the lymphatics and the mechanisms of tumor cell intravasation into the lymphatic vessels remain elusive. Conventional wisdom implies that tumor cells will be delivered into the sentinel lymph nodes with the flow of lymph once they are inside the lymphatic lumen, and this has indeed been demonstrated for tumor cell transport within large, collecting lymphatic vessels (Dadiani et al., 2006; Hayashi et al., 2007). In lymphatic capillaries, however, dendritic cells have been shown to crawl along the luminal side of LECs towards lymph node in the direction of flow (Tal et al., 2011), opening the possibility that tumor cells could employ similar mechanisms.

Subcapsular sinus (SCS) of the LN, which is lined by LECs, is the first site of lymph node metastasis (Carr, 1983; Carr et al., 1985; Dadiani et al., 2006; Das et al., 2013; Dewar et al., 2004). Dilation of SCS, which starts at the junction with the afferent lymphatic vessel, precedes arrival of tumor cells (Das et al., 2013) and may be a prerequisite for allowing the entry of tumor cells into the SCS. Indeed, in the absence of the primary tumor, when injected directly into the lymphatic system, osteosarcoma and melanoma cells arrest at the junction of the afferent lymphatic vessel and the LN (Hayashi et al., 2007). Scanning Electron Microscopy (SEM) analysis revealed that SCS is divided vertically and horizontally into smaller compartments, resulting in passages 5–15 μm wide (Das et al., 2013; Jia et al., 2012; Ohtani and Ohtani, 2008). Since the diameter of a single circulating tumor cell is at least 15 μm (Vona et al., 2000), it has been concluded that the small dimensions of the sinus prevent passive flow of tumor emboli into the SCS (Das et al., 2013). Chemokine CCL1 produced by the SCS LECs facilitates tumor cell entry into the open SCS as well as subsequent migration