Lymphology 39 (2006) 62-68

LYMPHANGIOGENESIS REVIEWS

THE RELATIONSHIP BETWEEN TUMORS AND THE LYMPHATICS: WHAT MORE IS THERE TO KNOW?

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ABSTRACT

Ten years ago the relationship between tumors and the lymphatic system was perceived to be rather passive. Since then, the dramatic increase in our understanding of the molecular biology of lymphatic endothelial cells and the regulation of lymphangiogenesis has revealed that tumors can actively interact with the lymphatics by inducing lymphangiogenesis. In turn, this interaction promotes the entry of tumor cells into the lymphatic vasculature and their subsequent transport to regional lymph nodes, a process that stimulates the formation of metastases. Tumor-induced lymphangiogenesis has thus emerged as an important new target in the fight against metastatic cancer. Nevertheless, there is still much to be learned about the relationship between tumors and the lymphatics that will have important ramifications for the design of clinical trials aimed at the application of anti-lymphangiogenesis therapies in the management of cancer. This Lymphangiogenesis Review *focuses on these issues.*

Keywords: lymphangiogenesis, cancer, metastasis, VEGF-C, VEGF-D, VEGFR-3, anti-lymphangiogenesis

The relationship between tumors and the lymphatic vasculature is of central importance to the pathology of human cancer. The vast majority of human cancers are carcinomas that have a predilection for metastasizing to regional lymph nodes. In many cases, metastasis to these lymph nodes is the first event in the onset of lethal dissemination of the cancer to vital organs [reviewed in (1)]. In order to be able to form metastases in regional lymph nodes, tumor cells from the primary cancer need to invade and be transported in the draining lymphatic vasculature to the regional nodes. Hence, it is vital to understand how tumors interact with the lymphatic vasculature if we are to be able to intervene effectively in metastatic disease.

Driven by the discovery of novel markers specific for lymphatic endothelial cells [reviewed in (2)], there have been rapid recent advances in understanding the biology of lymphangiogenesis, the new growth of lymphatic vessels (3). In turn, this has cast new light on the molecular and cellular basis of metastasis to regional lymph nodes (4). The majority of work has focused on the receptor tyrosine kinase VEGFR-3 that is virtually exclusively expressed on lymphatic but not blood endothelium in the adult. Activation of VEGFR-3 by its ligands VEGF-C and VEGF-D is sufficient to induce lymphangiogenesis (5). Correlative studies with human tumors and functional studies using animal tumor models show that increased levels of VEGF-C or VEGF-D in

tumors can lead to enhanced numbers of lymphatic vessels in the vicinity of tumors, which in turn promotes metastasis to regional lymph nodes by providing a greater number of entry sites into the lymphatic system for invading tumor cells [reviewed in (6)]. These findings identify tumor-induced lymphangiogenesis as a possible therapeutic target for the management of cancer and have prompted studies to investigate whether inhibitors of VEGFR-3 activation might represent novel therapeutic agents for the suppression of metastasis (7). Given the current excitement about possible clinical application, one might think that there is little more to discover in this field. However, as is often the case, the more that is discovered, the more open questions arise *(see Fig. 1)*. This *Lymphangiogenesis Review*, therefore, does not aim to give a synthesis of published results that have already been extensively reviewed, but rather to examine our current understanding of the tumor-lymphatic relationship with regard to key areas where further research is required to inform possible clinical application.

Human Tumors Exhibit a Variety of Relationships with the Lymphatics

Early studies suggested a rather simple and comparatively uniform relationship between tumors and the lymphatics, in which VEGFR-3 activation resulted in peritumoral lymphangiogenesis and promoted metastasis to regional lymph nodes by increasing the probability of tumor cell entry into the lymphatics (8). However, the subsequent explosion in the number of studies examining VEGF-C and VEGF-D expression in human tumors, together with analysis of lymphatic vessel morphology, proliferation status and density in the tumor context has revealed a more complex picture. First, not all studies find a statistically significant correlation between VEGF-C and VEGF-D expression and lymphatic density, regional lymph node metastasis formation or poor prognosis (6). The reasons for this are several-fold. First, if the primary tumor is located in a tissue that has a relatively high lymphatic vascular density, entry of invasive tumor cells into the lymphatics may occur efficiently in the absence of neo-lymphangiogenesis. Second, several studies provide evidence that tumors can co-opt pre-existing lymphatic vessels, again probably affording efficient entry of invasive tumor cells into the lymphatics [e.g., (9)]. Third, it is now becoming clear that while VEGF-C and VEGF-D are major regulators of lymphangiogenesis, a variety of other factors are also able to induce lymphangiogenesis (10). These factors include VEGF-A, hepatocyte growth factor (HGF) and members of the fibroblast growth factor, angiopoietin, platelet-derived growth factor and insulin-like growth factor families of secreted proteins. These potentially pro-lymphangiogenic factors have each been reported to be expressed in the context of tumors and therefore could contribute to tumor-induced lymphangiogenesis, although in most cases this remains to be demonstrated.

There is also now compelling evidence that lymphangiogenesis is not just a phenomenon that occurs in the stroma at the periphery of the tumor, but that it can also occur within the tumor [see (6)]. What regulates whether lymphangiogenesis occurs peritumorally, intratumorally or both is not clear. We also have no idea as to the relative importance of the peritumoral lymphatics compared to intratumoral lymphatics in terms of entry and transport of tumor cells into the lymphatics and subsequent lymph node metastasis formation, and the current literature contains contradictory findings (e.g., 11,12). This issue is further complicated by recent observations that suggest that macrophages can mimic lymphatic endothelial cells in the context of tumors (13).

Taken together, it is clear that we still have much to learn about the relationship between tumors and the lymphatics. These lessons will also be very important in the context of blocking tumor-induced lymphan-

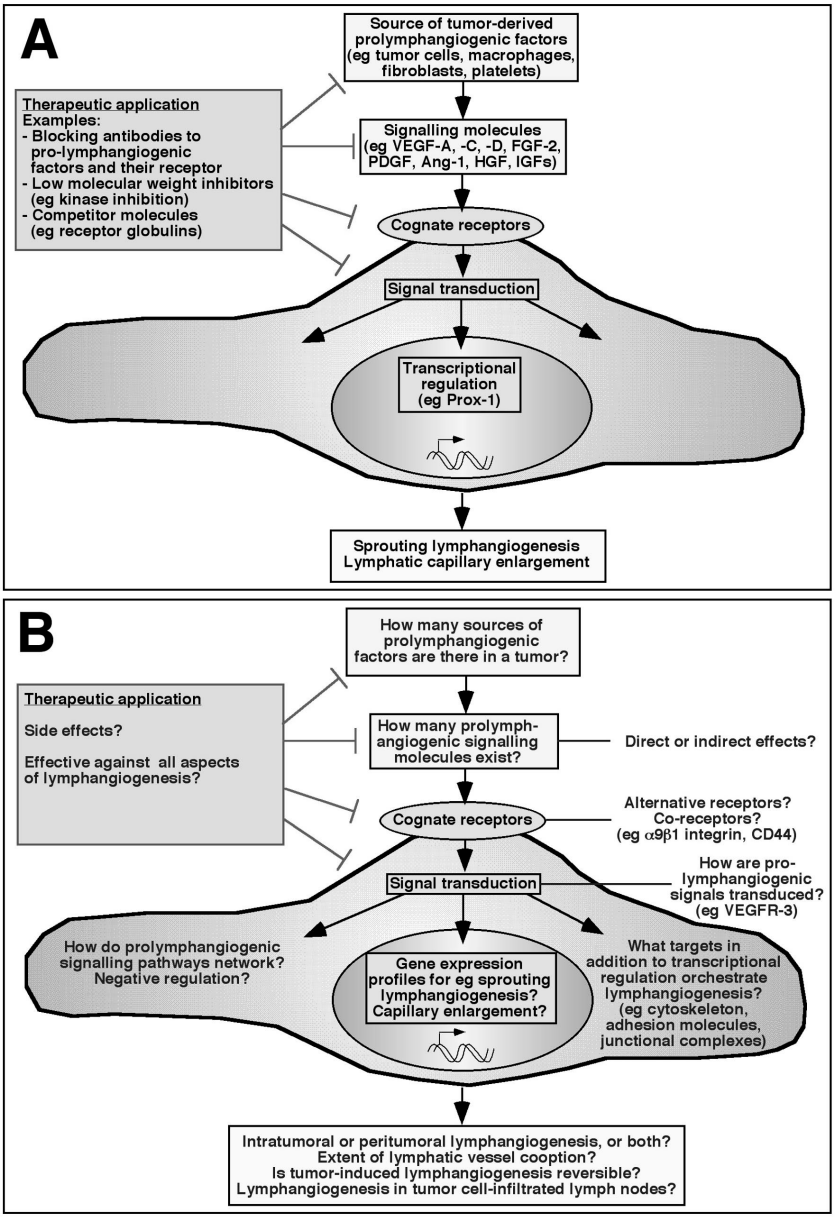

Fig. 1. Schematic diagram of a lymphatic endothelial cell summarizing the current status of our understanding of the regulation of tumor-induced lymphangiogenesis and attempts to block this process (A), and the important open questions that remain to be answered in these areas (B). See main text for details.

giogenesis in a therapeutic context. If multiple factors produced by tumors are able to induce enlarged or increased numbers of lymphatic vessels, then targeting a single one of these factors is unlikely to be effective. Furthermore, understanding the regulation and relative importance of vessel cooption, peri- and intratumoral lymphangiogenesis will have a profound impact on approaches to inhibit the interaction of tumors with the lymphatics.

Equally importantly, we still do not know whether tumor-induced effects on the lymphatics are reversible. If so, then therapies directed against the tumor-associated lymphatics will potentially have a broad application in trying to prevent the onset of metastasis. If not, then the onset of tumor-induced lymphangiogenesis in pre-invasive lesions (14), in recurring tumors and in developing metastases (15) may limit the efficacy of such approaches.

The Intra- and Extracellular Signaling That Regulates Tumor-induced Lymphangiogenesis Is Poorly Understood

To date, virtually all attempts to therapeutically inhibit tumor-induced lymphangiogenesis have focused on preventing the activation of VEGFR-3 expressed on lymphatic endothelial cells (LEC), either by blocking its interaction with its ligands, or by interfering with the function of its kinase domain. Approaches include the use of blocking antibodies or receptor globulins that inhibit the interaction of VEGFR-3 with its ligands, and low molecular weight chemicals such as indolinones that inhibit VEGFR-3 kinase activity (7). However, a fuller understanding of the intra- and extracellular signals that orchestrate lymphangiogenesis would likely identify additional targets and would also allow more efficient or precise inhibition of tumor-induced lymphangiogenesis.

The recent discoveries that growth factors in addition to those that activate VEGFR-3 can also act in a pro-lymphangiogenic manner (10) suggest that a variety of cell surface receptor-ligand interactions may represent targets for blocking tumor-induced lymphangiogenesis. Furthermore, it may be necessary to block more than one such interaction in order to prevent tumor-induced lymphangiogenesis. Additional complexity comes from the observation that the pro-lymphangiogenic effects of a given receptor-ligand pair may be direct or indirect. For example, HGF has been reported to directly promote lymphangiogenesis, but also to have indirect effects through promoting activation of VEGFR-3 (16,17). VEGF-A can also have direct and indirect effects (15,18,19). Furthermore, in addition to the cognate receptors, other cell surface molecules may regulate the effects of pro-lymphangiogenic ligands. Alternative receptors have been reported. For example, VEGF-C and VEGF-D are able to bind to and activate $\alpha 9\beta 1$, an integrin that has been implicated in the development of the lymphatic vasculature (20,21). Coreceptors may be also required. CD44, for example, has been shown to be required as a coreceptor for c-Met, the cognate receptor for HGF (22). In view of these findings it will be important to analyze in human tumors and animal models the spectrum of receptor-ligand interactions that can contribute to tumor-induced lymphangiogenesis, their relative importance to this process, and their mode of action. This will allow appropriate strategies for the inhibition of tumor-induced lymphangiogenesis to be developed.

With the recognition that a number of different growth factors have the capacity to induce lymphangiogenesis, it will be important to identify which intracellular signaling pathways these factors activate in order to exert their pro-lymphangiogenic effect. By studying the interaction and networking of the pathways that are activated by the different pro-lymphangiogenic factors, it may be possible to identify regulatory nodes that could be therapeutically targeted in order to block the effects of multiple pro-lymphangiogenic factors. Much remains to be discovered. For example, we are only just beginning to understand the signal transduction pathways that are activated and orchestrate lymphangiogenesis subsequent to the ligand-induced autophosphorylation of VEGFR-3. Recent work using blood vascular endothelial cells suggests that VEGFR-3 signals via the ERK, JNK and AKT pathways (23). This may also be the case in LEC but remains to be shown. Furthermore, attention to date has been

focused fairly exclusively on pro-lymphangiogenic signaling. Negative counter-regulation may exist, activation of which may also prove effective in blocking tumor-induced lymphangiogenesis.

The signal transduction pathways that are activated in response to pro-lymphangiogenic factors are likely to have a number of end-points, including transcriptional regulation. VEGF-C, for example, regulates expression of a number of genes (24). It is clear that the homeobox transcription factor Prox-1 plays an important role in regulating the expression of a profile of genes that determine aspects of LEC morphology and behavior (25,26). However, how Prox-1 is wired into the regulatory pathways that orchestrate lymphangiogenesis, what other transcriptional regulators play a role, and how different aspects of lymphangiogenesis (e.g., sprouting lymphangiogenesis compared to capillary enlargement) are regulated at the genetic level is not known. Other end-points of pro-lymphangiogenic signal transduction pathways are likely to include the cytoskeleton and adhesion complexes amongst others, but these remain to be identified.

A further approach that could conceivably inhibit tumor-induced lymphangiogenesis would be to block production of pro-lymphangiogenic factors in tumors. However, even for VEGF-C the picture is complicated, with tumor cells, stromal fibroblasts, tumor-associated macrophages and platelets all having been shown to act as sources of VEGF-C in the context of tumors [see (6)]. Given that a number of different factors may induce lymphangiogenesis in the context of tumors, each of which may have more that one cellular source, it becomes clear that inhibiting tumor-induced lymphangiogenesis by preventing production of pro-lymphangiogenic factors is unlikely to be a viable approach.

Will the Inhibition of Tumor-Induced Lymphangiogenesis Be Effective in Controlling Metastatic Cancer?

A major question remaining to be answered is the extent to which lymph node metastases contribute, if any, to metastasis formation in other organs. This question has exercised tumor biologists for decades [reviewed in (1)], and its answer will have a direct impact on the extent to which therapeutic inhibition of tumor-induced lymphangiogenesis is likely to be effective in the management of cancer. Lymph node metastases themselves are seldom life-threatening and can in the main be removed surgically if necessary. Inhibition of metastasis formation in regional lymph nodes by suppressing tumor-induced lymphangiogenesis will therefore only be effective if metastases in regional lymph nodes not only indicate that the primary tumor has gained metastatic competence, but also make a major contribution to tumor cell dissemination to vital organs, where subsequent impairment of function and destruction of these organs exerts the lethal effect of the cancer. A mechanism for the involvement of lymph node metastases in dissemination to vital organs is provided by the fact that lymph node metastases can shed tumor cells into the efferent lymphatics and thereby ultimately into the blood stream via the thoracic duct (1). The induction of lymphangiogenesis by metastatic tumors in lymph nodes that has been reported in some studies (15) may also play a role in this regard. Analysis of the metastatic process in animal models provides some but as yet inconclusive evidence of a role for lymph node metastases in dissemination to vital organs, and there is at best only indirect evidence in this regard from the study of human tumor progression [reviewed in (6)]. Clearly, more studies are required in this area.

A further consideration when weighing the potential efficacy of inhibition of tumor-induced lymphangiogenesis in the management of cancer is the possibility that normal physiological processes might be affected leading to unwanted side-effects. Studies in the adult organism suggest that lymphangio-

genesis only occurs significantly during wound healing and tissue regeneration. Inhibition of lymphangiogenesis in regenerating tissues could conceivably result in edema. In the context of wound healing, the newly-formed lymphatic vessels regress during wound resolution (27), and their function is poorly understood. Blocking the activity of pro-lymphangiogenic factors may also affect the function of cells other than LEC. VEGFR-3, for example, has been implicated in certain hematopoietic processes (28-30), and it is also expressed in neuronal cells such as the neuropil of the spinal cord, neuronal cells of the retina and other non-vascular cells in the cerebral cortex (31-33). This raises the possibility that targeting VEGFR-3 may have hematological or neurological consequences, although currently this remains speculative.

CONCLUSIONS

It is now clear that inhibition of tumor-induced lymphangiogenesis and suppression of the entry of tumor cells into the lymphatic vasculature represents a novel target for the management of cancer. However, there are a number of issues regarding the therapeutic potential of anti-lymphangiogenic treatments in the context of cancer that remain to be resolved. The spectrum and relative importance of molecules that induce lymphangiogenesis and the signal transduction pathways that they activate in LEC need to be defined. Furthermore, the relative importance of the different ways in which tumors interact with the lymphatics, the reversibility of tumor-induced lymphangiogenesis, and possible side effects of anti-lymphangiogenesis-based therapies all need to be investigated. Most importantly, the extent to which lymph node metastases contribute to the formation of metastases in other organs remains to be elucidated. These issues urgently need to be addressed so that clinical trials can be properly designed.

ACKNOWLEDGMENTS

This work was supported by a grant from the Deutsche Forschungsgemeinschaft to JPS under the auspices of SPP 1190 “The tumor-vessel interface.”

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