Lymphology 33 (2000) 144-147

EDITORIAL

LYMPHANGIOGENESIS AND TUMOR METASTASIS: MORE QUESTIONS THAN ANSWERS

The metastatic spread of tumor cells is responsible for the majority of cancer deaths, and with few exceptions, all cancers can metastasize. Clinicopathological observations suggest that for many cancers, transport of tumor cells via lymphatics is the most common pathway of initial dissemination. Many key questions regarding the mechanisms of lymphatic tumor spread still remain:

a) Does lymphatic tumor dissemination occur through pre-existing vessels, or does this require the de novo formation of lymphatic capillaries (lymphangiogenesis)?
b) What are the molecular mechanisms of lymphangiogenesis?
c) Is the process of tumor cell intravasation into lymphatics analagous to that which occurs in the blood vascular system?
d) Is inhibition of lymphangiogenesis a realistic therapeutic strategy for inhibiting tumor cell dissemination and the formation of metastasis?

The presence of tumor cells in peri- or juxta-tumoral lymphatics is not an uncommon feature in many primary tumors. It has long been suggested that "a lymphatic system as an anatomical entity is not demonstrable in tumors" (1), and studies in human and animal tumors involving injection of tracers into lymphatics revealed that tumors do not have an intrinsic lymphatic vascular supply (see for example 2,3). It has been proposed that this reflects the collapse of lymphatics within the tumor due to high interstitial pressure, and that this in turn further increases pressure within the tumor interstitium (4). Although the lack of intratumoral lymphatics appears to be a consistent feature, dilated and engorged lymphatics in peri-tumoral stroma, which occasionally penetrate into the tumor periphery, are features which are observed with equal frequency (4,5).

Does lymphatic dissemination of tumor cells require active tumor cell intravasation into lymphatic vessels in a manner analagous to that which occurs in the blood vascular system (6)? In a detailed study on breast carcinoma, Hartveit (5) has described the presence of an open lymphatic labyrinth in close association with the primary tumor, and suggests that "tumour cells lying free in the periductal lymphatic spaces will be washed with the tide of tissue fluid into the labyrinth through the sinuses and on into the lymphatic drainage channels. There is no need to postulate active lymphatic invasion". Evidence in favor of this hypothesis, or the alternative hypothesis of active tumor cell intravasation, is still lacking in the lymphatic system.

Lymphangiogenesis has traditionally been overshadowed by the greater emphasis placed on the blood vascular system (angiogenesis). This is due in part to the lack of identification of lymphangiogenic factors, as well as suitable markers with which to distinguish blood from lymphatic vascular endothelium. However, this scenario is changing rapidly following the discovery of the first lymphangiogenic factor, vascular endothelial growth factor-C (VEGF-C), which binds to VEGF receptors (VEGFRs) -2 and -3 (7,8). In in the normal adult organism,

VEGF-C appears to be a lymphangiogenic factor, and VEGFR-3 is restricted to lymphatic endothelium. VEGFR-2 in contrast is expressed both by blood vascular and lymphatic endothelium. However, VEGF-C also induces the formation of new blood vessels, but this appears to be restricted to early development and certain pathological settings such as tumorigenesis; in both of these settings, VEGFR-3 is also expressed by blood vascular endothelium (9,10).

The second major advance in the field of lymphangiogenesis has come with the discovery of lymphatic endothelium-specific markers. These include: podoplanin, a glomerular podocyte membrane mucoprotein (11); Prox-1, a homeobox gene product involved in regulating early lymphatic development (12); and LYVE-1, a lymphatic endothelial receptor for the extracellular matrix/lymphatic fluid glycosaminoglycan, hyaluronan (13). Although VEGFR-3 is expressed exclusively by lymphatics in normal adult tissues, the fact that it is widely expressed in embryonic blood vascular endothelium and is re-expressed in tumor blood vessels complicates its use in studies on tumor lymphangiogenesis.

A number of reports have recently described a correlation between VEGF-C expression, tumor lymphangiogenesis, and the formation of metastasis in regional lymph nodes. Thus, a significant correlation has been described in a variety of cancers (thyroid, prostate, gastric, colorectal and lung) between VEGF-C levels in primary tumors and lymph node metastases (14-19). One study has described a strong correlation between lymphatic vessel density and VEGF-C expression (20). However, in this study, no correlation was observed between lymphatic vessel density and lymph node metastases. Despite these highly suggestive correlative clinical findings, a direct role for VEGF-C in tumor lymphangiogenesis and subsequent metastasis has yet to be demonstrated, and to date there is no animal model in which these phenomena can be explored. In addition, these observations raise a number of questions regarding the mechanisms by which increased expression of VEGF-C in primary tumors results in an increase in lymph node metastases:

a) Does increased VEGF-C expression promote an increase in lymphatic vessel density?
b) If the answer is yes, is this sufficient to increase the rate of metastases to lymph nodes?
c) If not, are there non-lymphangiogenic functions of VEGF-C which account for the increase in lymph node metastasis? These might include the production of trophic, mitogenic or chemotactic factors for tumor cells by VEGF-C-stimulated lymphatic endothelium.
d) Are lymphatics passive conduits for tumor cells or can their adhesive and transport capacities be modulated?

Despite the finding that increased expression of VEGF-C in spontaneously arising human tumors correlates with dissemination of tumor cells to regional lymph nodes, it is not known whether pre-existing lymphatics are sufficient to serve this function, or whether metastasis requires the de novo formation of lymphatic capillaries (lymphangiogenesis). Some authors have suggested that it is not necessary to invoke lymphangiogenesis in this setting, and that pre-existing peritumoral lymphatics will suffice (4).

Numerous attempts have been made since 1984 (21,22) to isolate and culture endothelial cells from lymphatic vessels in a variety of species (human, bovine, canine, ovine, rat, murine). However, almost all previous studies describe the isolation of cells from mesenteric collecting or thoracic ducts, i.e. they are of large vessel origin. In a 3-D collagen gel model of in vitro angiogenesis (23), bovine large vessel lymphatic endothelial cells (LECs) were induced to invade in response to VEGF, VEGF-C and basic fibroblast growth factor (bFGF), and to form capillary-like tubular structures; in this assay, synergism was observed between bFGF and VEGF (24,25). (Hem)angiogenesis (and

presumably lymphangiogenesis) is characterized by the triad of endothelial proliferation, migration and protease activity. With respect to proliferation, this phenomenon is regulated positively in bovine large vessel LECs by bFGF, epidermal growth factor and transforming growth factor-alpha, whereas tumor necrosis factor-alpha (TNF-α) and interleukin-1 are inhibitory (26). With respect to migration, bFGF stimulates, whereas TNF-α inhibits migration in bovine large vessel LECs (26). Finally, with respect to protease activity, VEGF, VEGF-C and bFGF stimulates expression of urokinase-type plasminogen activator (uPA), uPA receptor, tissue-type PA (tPA) and PA inhibitor-1 (PAI-1) in bovine large vessel LECs (24-26). tPA and PAI-1 are also stimulated by TNF-α (26,27).

As indicated, all previous in vitro studies have been performed on large vessel LECs. However, postnatal lymphangiogenesis occurs by sprouting from pre-existing lymphatic capillaries (28,29). In the future, it will therefore be of paramount importance to work with primary endothelial cells from lymphatic capillaries. In addition to in vitro studies similar to those described above, lymphatic capillary endothelial cells would be useful (a) for the identification of molecules involved in adhesive interactions with other cells (e.g.: lymphocytes, tumor cells); and (b) for application of techniques of differential gene expression to identify molecular differences between blood and lymphatic capillary endothelial cells. The utility of these techniques in identifying gene expression profiles in normal versus tumor endothelial cells has recently been demonstrated (30).

Finally, an extensive effort is currently being directed world-wide to identify anti-angiogenic agents, particularly for use in anti-cancer therapy (31-34), and many potentially useful compounds have progressed beyond pre-clinical studies into the early phases of clinical trials. The recent, almost explosive interest in lymphangiogenesis, after many decades of dormancy, will undoubtedly ensure that we move rapidly to attain similar objectives in the lymphatic system. However, it still remains to be determined whether inhibition of lymphangiogenesis is a realistic therapeutic strategy for inhibiting tumor cell dissemination and the formation of metastases. Likewise, we still lack a relevant animal model in which the mechanisms of lymphangiogenesis and lymphatic tumor metastasis can be dissected, and in which potential inhibitors of these processes can be tested.

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