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Lymphatic or Hematogenous Dissemination: How Does a Metastatic Tumor Cell Decide?

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Abstract

The formation of distant metastases is the deadliest phase of cancer progression. Although numerous studies have identified genes and mechanisms that affect metastasis after tumors have reached secondary sites, our knowledge about how cancer cells initially gain access to systemic circulation is limited. Since tumors can enter the blood directly by intravasating into venous capillaries or indirectly via lymphatics, it is important to evaluate the relative contributions of both pathways as routes of egress from the primary site. Insights into tumor and stromal factors governing the intravasation process may help explain why certain tumors exhibit "preferred" pathways for metastatic dissemination, both clinically and in experimental animal models.

Keywords

metastasis; intravasation; angiogenesis; lymphangiogenesis

WHICH TUMORS METASTASIZE?

What makes a tumor cell metastatic? Certainly, proliferative ability at a distant site is essential for metastasis (Paget's "seed and soil" hypothesis), and difficulties in establishing secondary growth might explain why fewer than 0.01% of circulating tumor cells actually form metastases.[1–3] However, exactly what enables a cancer cell to complete the metastatic process is not entirely clear. While recent gene expression studies have suggested that distant metastases resemble their primary tumors of origin,[4,5] other studies have indicated that the expression of specific genes is altered in metastatic cells.[6–8] A model combining both these observations has speculated that cells derived from metastases and from their corresponding primary tumors share an overall gene expression signature that confers the ability to complete some, but not all, of the steps required for metastasis.[7,9] On top of this, the altered expression of a limited number of additional genes may render a sub-population of cells fully competent for metastasis, without changing its overall similarity with the primary tumor.

Although metastasis is widely regarded as an inefficient process, most cancer patients die from metastases rather than from their primary tumors. Metastatic inefficiency is likely overcome by the sheer number of tumor cells that enter the systemic circulation daily, estimated in one study to be up to ~4×10^6 tumor cells released per gram of primary tumor.[10] Consequently, it is important that we gain a detailed understanding of how tumors complete the earliest steps of metastasis, including intravasation into vasculature.

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In order to metastasize, cancer cells must first detach from the primary tumor and invade blood vessels or lymphatics. This may be a passive process where cells are simply sloughed off from the primary tumor or an active one involving directed migration.[11,12] Almost certainly, a tumor's cell of origin and its accompanying differentiation program will affect its metastatic proclivity.[13] Cells from connective tissue tumors such as fibrosarcomas and gliomas tend to migrate individually, for instance, whereas those from melanomas and carcinomas often migrate collectively.[14] In addition, highly differentiated epithelial tumors may initially display collective migration, only to de-differentiate and exhibit single cell invasion, a process termed epithelial-to-mesenchymal-transition (EMT).[15] Indeed, genes that promote EMT—including Twist;[8] Slug and Snail transcription factors;[16] and components of the TGF-β signaling pathway[17,18]—have all been reported to enhance the earliest stages of metastasis. E-cadherin, which is often lost during EMT, is thought to suppress cell migration and tumor progression.[19] Finally, stromal cells such as fibroblasts and macrophages have also been reported to affect metastasis by contributing growth factors (e.g., EGF, FGF-1), matrix metalloproteinases and chemotactic/pro-migratory factors (e.g., SF/HGF, chemokines).[12,14]

BLOOD VESSEL OR LYMPHATIC DISSEMINATION?

Once a migratory cell(s) has detached from the primary tumor, it may intravasate into blood vessels or lymphatics. Either route of dissemination can lead to venous circulation, as lymphatics drain into blood, most commonly through the left lymphatic duct (thoracic duct) or the right lymphatic duct, and then subsequently into the subclavian veins. Along the way, lymphatic fluid is filtered by lymph nodes.

In the absence of overt metastases, hematogenous dissemination of tumors is assayed by detecting cancer cells in the peripheral blood of patients or from bone marrow aspirates.[20] The presence of circulating tumor cells and micrometastases can be determined by RT-PCR or immunohistochemistry (IHC), particularly for cytokeratins in the case of epithelial tumors. Lymphatic spread is also assayed by IHC and/or RT-PCR following surgical removal of regional lymph nodes. Tumors almost invariably invade lymph nodes in sequence, starting with the nearest (sentinel or draining) node, followed by increasingly distal ones.[21] If the draining lymph node is uninvaded, other lymph nodes are also likely free of metastases.[22]

Metastatic bias is illustrated by the fact that carcinomas and melanomas tend to develop lymph node metastases more frequently than sarcomas,[14] although it is unclear whether this disparity is due to differences in intravasation and/or growth. Lymph nodes are often the first site of metastasis in a variety of cancers, and are critical for tumor staging and prognosis.[22] In prostate cancer, for instance, 75% of patients bearing lymph node metastases at the time of diagnosis will possess bone metastases within 5 years, regardless of treatment.[23] The presence of tumor cells in the bone-marrow is also predictive of distant metastases in a variety of tumors, particularly carcinomas.[20] On the other hand, the prognostic value of circulating tumor cells in the blood is debated, as current techniques for detection suffer from problems such as low sensitivity and high rates of false positives.[24,25] However, recent studies using an automated platform for detecting tumor cells in the blood, called CellSearch, have reported significant correlations between the presence of circulating tumor cells and poor clinical outcome for breast cancer patients.[26]

The decision to intravasate into either blood or lymphatic vessels may rest largely on physical restrictions imposed on invasive tumors, although active mechanisms for attracting cells to specific types of vasculature have also recently been proposed (see below). Lymphatic capillaries lack the tight interendothelial junctions typically seen in blood vessels, as well as the surrounding layers of pericytes/smooth muscle cells and basement membranes.[27] This inevitably renders lymphatics "leaky" relative to blood vessels, thus lowering the barriers for

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tumor intravasation. In addition, tumor cell survival may benefit from the passive, low-shear system of fluid transport characteristic of lymphatics.

Accessibility of blood and lymphatic vasculature may also influence the pathway taken for metastasis. Induction of angiogenesis, the growth of blood vessels, has been shown to be necessary for tumors growing beyond 0.4 mm in diameter.[28,29] Lymphangiogenesis, the growth of lymphatic vessels, has been inhibited by us[30] and others[31–37] in experimental mouse cancer models without affecting primary tumor growth. Because blood and lymphatic vessels share a common embryonic origin, and respond to many similar growth factors—VEGF-A, VEGF-C, VEGF-D, FGF2, PDGF-B, HGF and others[38]—tumors might be expected to induce lymphangiogenesis concomitant with angiogenesis. But for reasons unclear, this is often not the case. While proliferating intratumoral lymphatics have been detected in human melanomas,[39] as well as in head and neck squamous cell carcinomas,[40] evidence for lymphangiogenesis in other cancers has been less well documented. The presence of anti-lymphangiogenic factors may be one reason why proliferating intratumoral lymphatics are not more commonly found in human clinical tumors,[41] though the identity of these proposed factors is currently unknown.

Nonetheless, intratumoral lymphatics may provide a possible escape route from the primary tumor to draining lymph nodes, and indeed, several studies have reported that inhibition of lymphangiogenesis in xenograft tumor models can significantly reduce lymph node metastasis.[31–33,36] However, other studies have suggested that intratumoral lymphatics are compressed and nonfunctional.[42–45] This apparent absence of functional intratumoral lymphatics would imply that tumor cells intravasated into these vessels will encounter blockages and dead ends that actually impede metastasis. The fact that many tumors metastasize to local lymph nodes despite absence of lymphangiogenesis or functional intratumoral lymphatics, has led some to propose that it is the peripheral, peritumoral lymphatics that mediate tumor cell dissemination.[37,46] We recently obtained results consistent with this hypothesis by selectively ablating intratumoral, but not peritumoral, lymphatics in a prostate cancer orthotopic model and showing that lymph node metastasis was not significantly altered.[30] Other studies that ablated peritumoral lymphatics or inhibited their “activation”—local vessel sprouting, dilation and permeability—were successful at reducing metastasis (Fig. 1).[32,37,47] It is possible that the studies reporting metastatic inhibition associated with ablation of intratumoral lymphatics[31–33,36] may actually reflect interference with tumor cell intravasation into peritumoral lymphatics. Recent clinical and spontaneous animal tumor studies have also reported that prostate,[48] breast[49,50] and pancreatic tumors[51] develop lymphatic metastases in the absence of intratumoral lymphangiogenesis.

Perhaps the best approach for studying blood and lymphatic vessel intravasation is to observe the process in real time, using in vivo intravital microscopy. Wyckoff et al imaged rat mammary adenocarcinomas and discovered that metastatic cells were more likely to polarize towards blood vessels than were nonmetastatic cells.[11,52] Interestingly, polarization of metastatic cells was explained by increased expression of EGF receptor, which made the cells chemotactic to EGF released by macrophages lining blood vessels. Furthermore, individual metastatic cells were seen intravasating into blood vessels using an amoeboid form of movement. Nonmetastatic cells, however, often fragmented upon crossing endothelial junctions. Consequently, the authors speculated that intravasation was a rate-limiting step for metastasis.

Although this work dealt with a limited number of established cell lines and did not examine lymphatics, it does raise several important considerations about the intravasation process. These considerations include the mode of cell migration utilized (individual amoeboid or fibroblastic movement, versus collective sheet/nest migration); the role of stromal cells in promoting polarized movement; and the effect of hemodynamic shear forces on cell viability

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—all of which may influence a tumor's preference for disseminating via blood vessels or lymphatics.

The type of cell movement undertaken is affected, in large part, by the surrounding extracellular matrix (ECM) and by the integrity of cell-cell junctions.[14] Mesenchymal, or fibroblast-like, single cell migration tends to occur when mature, integrin-containing focal contacts develop in the presence of dense matrix networks. Amoeboid migration is favored under less adhesive conditions, as is often seen in vivo or in three-dimensional cultures, when focal contacts are lacking.[53] The speed of amoeboid migration is about 10–30 times faster than mesenchymal migration and is protease-independent.[14,53] Given that lymphatic vessels lack basement membranes, and that ECM networks are likely less dense around peritumoral lymphatics than around intratumoral blood vessels, this would seem to favor rapid and efficient amoeboid-type intravasation into lymphatic circulation. Lymphatic permeability may also allow passage of cell aggregates that have retained expression of homotypic cell-cell adhesion receptors such as cadherins.[54]

In addition, active recruitment of tumor cells towards lymphatics may occur via EGF-EGFR-mediated chemotaxis, since macrophages have been found in proximity to lymphatic vessels.[55,56] In one study, macrophages were even reported to transdifferentiate into lymphatics in response to inflammation in an eye cornea model,[57] though the generality of this finding remains to be determined. Lymphatic stromal cells have also been reported to be a source of EGF and IGF-I.[58] In addition, lymph node secretion of chemokines such as SCL/CCL21 and CCL1 may attract tumor cells that express the receptors CCR7 and CCR8, respectively.[59] Overexpression of CCR7 in B16 melanoma cells has been shown to increase lymph node metastasis,[60] and others have reported that breast cancer cells or melanomas expressing CXCR4 may actively home to lymph nodes containing CXCL12/SDF-1 ligand.[61] Activated cancer-associated fibroblasts may also secrete chemokines that enhance tumor growth and invasion.[62]

Lastly, although intravasation into lymphatics may seem to be favored due to reduced shear stress inflicted upon the cell, increased hemodynamic flow rate may also help dislodge individual cells from the primary tumor. Disaggregation of cells under flow has been reported to be affected, at least in part, by levels of E-cadherin expression.[54]

HOW DO TUMOR CELLS REACH SYSTEMIC CIRCULATION?

Viable tumor cells have been isolated in the blood of patients bearing nearly all types of cancer, including the most common forms of carcinomas.[63] Although the amount of time a tumor cell spends circulating throughout the body is believed to be short, the sheer number of cells potentially available for seeding distant metastases makes it imperative for us to understand how tumors gain initial access to systemic circulation.

In many clinical studies involving different human tumors, a positive association between lymphatic and hematogenous metastasis has generally been observed. For instance, Bubendorf et al reported that 84% of patients with node-positive prostate cancer bore evidence of hematogenous dissemination, as opposed to 16% of patients without local lymph node spread.[64] In breast cancer, lymph node metastasis has been linked with poor prognosis and distant metastasis,[65] and similar observations have also been noted in pancreatic cancer,[66] ovarian cancer,[67] and head and neck cancer,[68] among others.

Recently, we observed a strong correlation between lymphatic and hematogenous dissemination in a mouse orthotopic xenograft model of prostate cancer (Fig. 2).[30] As expected, the lymph nodes directly draining the prostate, the para-aortic/sub-lumbar lymph nodes, were invaded first by the tumors, followed by the more distant sub-renal lymph nodes.

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Mice that bore tumors which had not formed macroscopic metastases in the lumbar lymph nodes (~50% of mice) also did not possess renal lymph node macro-metastases. Not surprisingly, the appearance of lung micrometastases was well-correlated with the detection of viable circulating tumor cells in the blood. Interestingly, however, significant numbers of lung metastases and circulating cells were found only in mice that possessed both renal and lumbar lymph node metastases, regardless of primary tumor size.

These clinical and experimental correlations can be interpreted in at least a couple of ways (Fig. 3). It is possible that some tumors may be unable to intravasate directly into blood vessels; thus they must establish satellite lymph node metastases first to disperse metastatic cells via the thoracic duct. Another possibility is that the primary tumors may be completely noninvasive until somehow triggered to metastasize via both lymphatics and blood vessels simultaneously. Either possibility would potentially yield an apparent correlation between lymphatic and hematogenous spread. But in the case of human patients, those with node-positive tumors at the time of diagnosis might be free of distal metastases in the first scenario but not in the second.

In support of the former possibility, Sleeman has noted that the physiology of lymph nodes may actually favor formation of local metastases that could serve as “bridgeheads” for further dissemination.[69] The low shear flow of lymphatic fluid coupled with the filtering of cells into a confined space—the subcapsular sinuses—may increase the local concentration of tumor cell aggregates in the node. This would be in contrast to the “scatter-shot” dispersal of individual tumor cells into large capillary beds such as the lung, where metastatic progression after seeding is known to be highly inefficient.[1] Indeed, increased cell aggregation has been previously found to enhance formation of experimental metastases.[70] Furthermore, according to Sleeman, tumor cells that have arrived in the subcapsular sinuses would not need to extravasate.[69]

Others have proposed that lymph nodes may act as initial “selection” sites where tumor cells with partial metastatic competence could seed and expand, while selecting for increasingly malignant variants that could later spread to more distant sites.[32] This would agree with hypotheses previously set forth that metastatic cells are similar to, but also different from, their primary tumors of origin.[7,9]

If entrance into systemic circulation were dependent on lymphatics, experimental inhibition of lymph node metastasis should also inhibit hematogenous spread. But, while some have indeed reported such results,[32,34,36] others found that inhibiting lymph node metastasis had no effect on lung metastasis.[31,37,71] These findings are likely attributable to differences in the cell lines utilized and whether the cells were implanted orthotopically or ectopically. In another study, resection of MT-100-TC mammary carcinomas along with draining lymph nodes prevented metastatic recurrence, but removal of the primary tumors alone did not.[72] This would suggest that MT-100-TC cells reached systemic circulation via lymphatics, a progression the authors termed “metachronous seeding.”

In contrast, the presence of hematogenous metastases in the absence of lymphatic spread would clearly indicate direct dispersal of tumor cells into blood vessels. This is a likely scenario for patients harboring bone marrow micrometastases in the absence of other detectable signs of spread, which has been reported to occur in 20–40% of carcinomas.[73] Interestingly, comparative genomic hybridization (CGH) analyses have suggested that malignant cells may disseminate through the blood very early in breast cancer.[74,75] These cells were also found to be distinct from lymph node metastases by CGH, thus arguing against metachronous seeding.[74]

Lastly, in patients harboring both lymphatic and hematogenous metastases, assessing the order of events remains difficult. One possible experimental approach to determine whether distant metastases arise directly from the primary tumor or indirectly from lymph nodes might be to

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construct a detailed time course tracking the relative temporal appearance of tumor cells in the blood and lymph nodes. If hematogenous spread occurs via lymphatics, for instance, malignant cells should appear in lymph nodes before blood. Such an approach could be coupled with methods such as CGH[74] or gene expression profiling[76] to track dispersed tumor cells. Detailed genomic analyses comparing primary tumors with micrometastases isolated from lymph nodes and/or distant sites should be able to distinguish the pathways undertaken for metastasis.

CONCLUSIONS

A confluence of factors likely influences whether primary tumors metastasize via blood vessel or lymphatic routes and, related to that, how tumor cells reach the systemic circulation. Differentiation programs innate to the cell of origin of each tumor may predetermine the metastatic phenotype, though additional genetic or epigenetic changes may also affect a cell's ability to intravasate. Morphological differences between blood vessels and lymphatics will almost certainly affect the initial route of spread, and in this regard, peritumoral lymphatics might be considered a default pathway for tumors incapable of crossing blood endothelial boundaries. However, active mechanisms for attracting tumor cells towards one type of vasculature versus another cannot be discounted. In addition, the roles played by inflammatory[77] and host hematopoietic precursor cells[78] in affecting the process will need to be further examined.

At the same time, improved imaging techniques should allow simultaneous visualization of blood vessel and lymphatic intravasation within the same tumor, allowing direct measurements of the relative frequencies of each occurrence. In addition, genomic approaches combined with clustering algorithms should be able to elucidate molecular relationships between disseminated tumor cells and cells derived from the primary tumor and/or lymph node metastases. These studies will likely yield detailed information about how and when metastatic cells leave the primary tumor. Lastly, identification and validation of genes and proteins that affect the intravasation process and perhaps specify whether a tumor invades via blood vessel or lymphatic routes, as has been recently proposed,[79] will have valuable clinical implications for prognosis and treatment.

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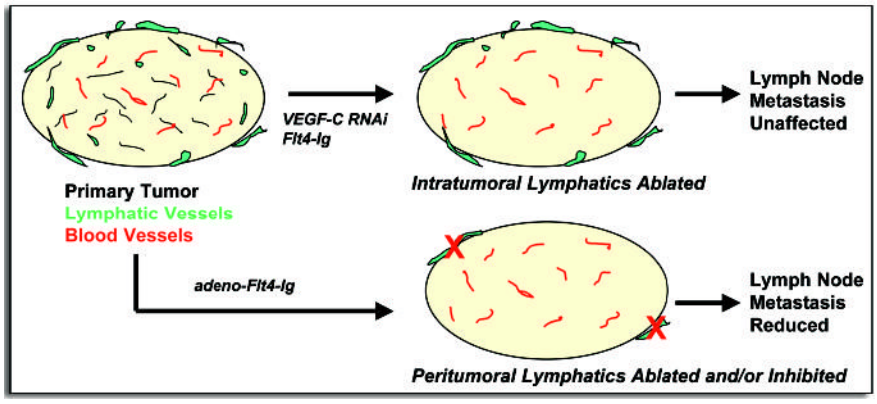

Figure 1.

Tumors possess blood vessels (red) and, in some cases, lymphatics (green). Experimental ablation of intratumoral lymphatics does not inhibit lymph node metastasis (top).[30] Eliminating or inhibiting the activation of peritumoral lymphatics has been shown to reduce lymphatic spread (bottom).[32,37,47] In addition, intratumoral lymphatics are absent in many tumors that nevertheless metastasize to lymph nodes.[51] These observations imply that peritumoral lymphatics mediate the majority of tumor cell dissemination. (Flt4-Ig, soluble Flt4 receptor/VEGFR3; adeno, adenoviral delivery).

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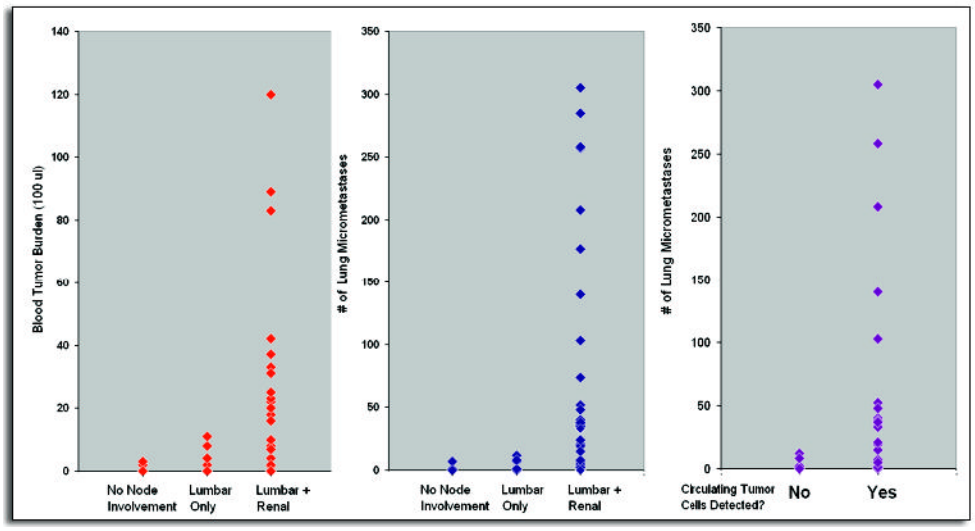

Figure 2.

After surgical orthotopic implantation of human prostate PC-3 cells into nude mice, associations were observed among lymph node metastasis, circulating tumor cells and lung micrometastases. *Left*, significant numbers of circulating tumor cells in the blood were detected only in mice that bore macrometastases in both the lumbar and renal lymph nodes. *Middle*, similarly, most lung metastases were seen in mice with both lymph node sites invaded. *Right*, lung metastases were correlated with the presence of circulating tumor cells in the blood. (Reproduced with permission from ref. [30]).

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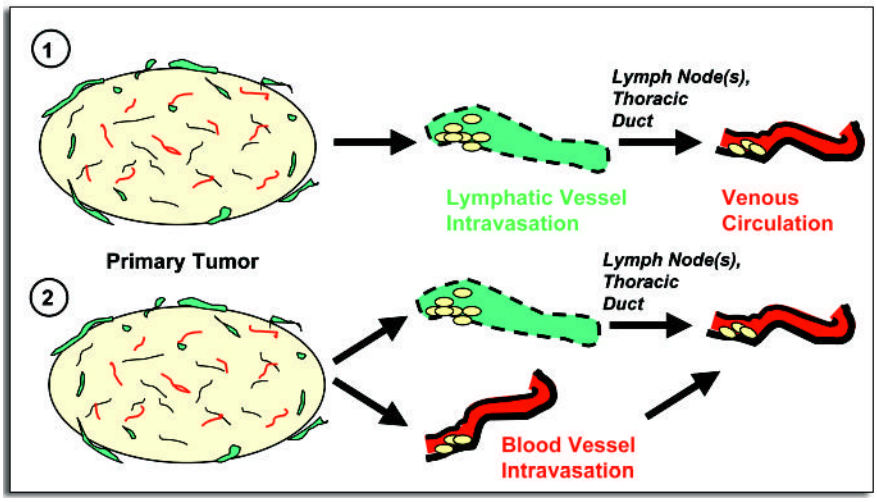

Figure 3.

Two possible pathways for metastasis could explain why, in a mouse model of prostate cancer, hematogenous spread is observed only in the presence of significant lymphatic spread. (1) The tumors might be incapable of intravasating directly into blood vessels, so metastatic cells enter venous circulation indirectly via lymphatics (“metachronous seeding”). Or, the tumor is completely nonmetastatic until mobilized to metastasize via both lymphatic and hematogenous routes at the same time (2).