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The lymph node microenvironment and its role in the progression of metastatic cancer

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Abstract

Lymph nodes are initial sites for cancer metastasis in many solid tumors. However, their role in cancer progression is still not completely understood. Emerging evidence suggests that the lymph node microenvironment provides hospitable soil for the seeding and proliferation of cancer cells. Resident immune and stromal cells in the lymph node express and secrete molecules that may facilitate the survival of cancer cells in this organ. More comprehensive studies are warranted to fully understand the importance of the lymph node in tumor progression. Here, we will review the current knowledge of the role of the lymph node microenvironment in metastatic progression.

Keywords

Lymph node; metastasis; lymphatic vessels; chemokine; clonality; lymphangiogenesis

1. Introduction

Cancer metastasis is a complex, multi step process that involves the dissemination of cancer cells from the primary site to distant organs [1]. Several recent studies have advanced our knowledge of the spread of tumor cells via the blood stream, however, less is known about the process of lymphatic metastasis. Mounting evidence suggests that lymphatic metastasis is not an entirely passive process as previously hypothesized, but is regulated at multiple steps including the transit of tumor cells via the lymphatic vessels and the successful seeding in draining lymph nodes [2]. The presence of tumor cells in regional or sentinel lymph nodes (SLN) has important clinical significance as it is associated with disease progression, poor prognosis and often determines the choice of therapies [3–5]. The increasing recognition of the importance of lymph node metastasis in cancer biology has prompted recent studies to unravel the molecular signals and cellular changes involved in this complex process. Some of these changes include lymphangiogenesis, the expansion of immunosuppressive cells, up-regulation of chemokines and cytokines, and blood vessel remodeling in the lymph node.

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These events help facilitate tumor cell entry, colonization and survival in the lymph node. However, the ultimate fate of tumor cells in the lymph node is still controversial [6].

Several studies have elucidated how tumor cells are attracted to the lymph node by chemokine signals that are often secreted by stromal or immune cells in the lymph node microenvironment [7]. In addition to attracting tumor cells to the lymph node, chemokines play an important role in modulating immune or stromal cell activity. Furthermore, the role of lymphatic vessels and lymphangiogenesis in altering the local inflammatory response and promoting an immunosuppressive microenvironment in the lymph node is critical for tumor cell dissemination to this organ [8]. Recent progress in lymphatic vessel biology, including the development of sophisticated intravital imaging techniques for monitoring the process of lymphangiogenesis and lymphatic metastases has led to the appreciation of the lymphatic system as a major contributor to cancer progression [9–15]. In this review we discuss recent progress in our understanding of the mechanisms involved in lymph node metastasis and the potential role of the lymph node in facilitating tumor cell proliferation and metastases to distant organs.

2. The lymph node, lymphatic vessels and lymphangiogenesis

The lymphatic vasculature is a series of vessels throughout the body that play important roles in tissue homeostasis, fluid balance, immune function, absorption of dietary fat and lipid transport. In addition to their role in normal physiology, lymphatic vessels play critical roles in pathological conditions including inflammation and cancer [16, 17]. Lymphatic endothelial cells express several molecular markers that include LYVE-1, Prox1, podoplanin (gp38), VEGFR-2, VEGFR-3, neuropilin-2, angiopoietin-1 and CCL21. In peripheral tissues, initial lymphatic vessels are thin-walled vessels that absorb fluid and cells from tissues. These vessels are porous and easily accessed by cells as they have overlapping endothelial micro-valves, intermittent basement membrane and lack pericyte and smooth muscle coverage [18]. Initial lymphatics carry lymphatic fluid into deeper pre-collecting vessels, then into larger collecting lymphatic vessels and finally back into the bloodstream [19]. In pathological conditions including cancer, lymphatic vessels and lymph nodes participate in disease progression and undergo remodeling in response to growth factors, chemokines and other signaling molecules secreted by tumor cells or other cells in the tumor microenvironment [17].

2.1. Sentinel lymph nodes: A niche for tumor growth

Tumor cell metastasis to distant sites is often preceded by changes in the microenvironment of the metastatic organ in preparation for the arrival and successful colonization of tumor cells [20]. The crosstalk between the tumor cell ('the seed') and the microenvironment ('the soil') was observed and described by Stephen Paget in 1889 and came to be known as the 'Seed and Soil' hypothesis [21]. Many decades later, we are beginning to uncover the cellular and molecular changes in the metastatic lymph node microenvironment prior to the arrival of tumor cells. Several studies have elucidated some distinctive features of a pre-metastatic SLN including increased lymphangiogenesis and lymph flow [22], remodeling of high endothelial venules (HEVs) [23, 24], recruitment of myeloid cells and diminution of effector lymphocyte count and/or function [25] (Figure 1). In mouse models of cancer as

well as in human patients, the HEVs in the pre-metastatic SLN have decreased vessel-wall thickness and increased cross-sectional vessel diameter that transition into thin-wall enlarged blood vessels [23, 24, 26]. The proximity of SLNs to the tumor may lead to an increased concentration in lymph-transported molecules that orchestrate these events. As tumor cells metastasize to lymph nodes or other tissues, distant lymph nodes may also undergo changes. Immune cell composition can be altered in tumor-free, non-SLNs, suggesting that SLN status is not required for changes to occur [27]. SLN lymphangiogenesis, but not angiogenesis, was also found to be a strong predictor of further lymph node metastasis [28].

In addition to the remodeling of the vasculature, SLN undergo expansion of immune cell and stromal cell populations and appear to have a largely immunosuppressive cytokine environment. Several cytokines have been found to play a prominent role in the immunosuppression of SLNs. In several studies IL-10, a potent immunosuppressive cytokine, was increased in SLNs relative to non-SLNs [29, 30]. Interestingly, IL-10 production was higher in tumor-free SLNs compared to lymph nodes with tumor [30, 31]. Other immunosuppressive cytokines and molecules such as TGF- β , GM-CSF, and PGE₂ from primary tumor and/or lymph nodes can influence the immune status of the SLN [25, 32, 33]. The SLNs become increasingly immunocompromised with an increase in primary tumor size [32]. It is possible however that both the primary tumor and SLNs are sources of immunosuppressive cytokines. It is also believed that recruitment of myeloid immune cell populations from the blood circulation in a premetastatic setting will promote an immunosuppressive microenvironment that facilitates the growth and expansion of tumor cells at that site [27]. Recent studies suggest that CD8⁺ T cells are capable of killing myeloid cells in SLNs as a potential mechanism to prevent colonization of tumor cells [34]. Taken together these studies support the phenomenon of a premetastatic niche in SLNs that is initiated by the primary tumor prior to the physical presence of metastatic cancer cells in the SLN.

2.2. Molecular signals precondition the lymph node microenvironment

The most well documented aspect of the premetastatic niche in SLNs is lymph node lymphangiogenesis driven by VEGF-A or VEGF-C [35, 36], which also correlates with increased distant metastases in these studies. VEGF-C-PI3K mediated signaling is important for remodeling the lymph node prior to tumor cell arrival by stimulating LEC proliferation, leading to tumor-associated lymphangiogenesis. This signaling leads to activation of integrin $\alpha 4 \beta 1$ on lymph node lymphatic endothelium, which attracts vascular cell adhesion molecule 1 (VCAM-1) expressing tumor cells, thereby promoting lymph node metastasis [37]. Erythropoietin (EPO) was also shown to act directly on lymphatic endothelial cells, enhancing lymph node lymphangiogenesis [38] and tumor metastasis. EPO also increased VEGF-C expression in inflammatory macrophages in draining lymph nodes. Similar to other studies of inflammation in non-lymphoid tissue, multiple growth factors may lead to lymphangiogenesis directly, or indirectly via up-regulation of VEGF-C.

Several studies have shown that high endothelial venule-associated molecules are down-regulated in the pre-metastatic lymph node. Specifically, loss of bone morphogenetic

protein-4 (BMP-4) in HEVs was associated with HEV remodeling [39], suggesting that BMP-4 is an inhibitor of lymph node vascular remodeling. Loss of peripheral node addressin (PNAd), an L-selectin ligand on the surface of HEVs that play an important role in lymphocyte homing, was observed in metastatic lymph nodes [24]. These findings suggest that primary tumors can remodel HEVs in SLNs to inhibit immune responses while providing any arriving cancer cells with nutrients through sustained blood flow.

In addition to effects on lymphatic endothelium, molecular signaling also regulates the immune cell populations in SLNs. By unknown mechanisms, distal tumor cells cause the loss of lymph node CCL21, impairing lymphocyte homing [40]. In contrast, Stat-3-S1PR1 signaling in myeloid cells from tumor free lymph nodes in cancer patients was increased [41]. Blockade of this pathway in animal studies led to a decrease in the incidence of metastasis. Another recent study by Ogawa and colleagues [25] demonstrated that dendritic cells (DC) in the sub-capsular sinus (SCS) of a premetastatic lymph node produced COX-2-derived prostaglandin E₂ (PGE₂). PGE₂ stimulated the EP3 receptor on DCs and up-regulated the expression of stromal cell-derived factor-1 α (SDF-1 α) as well as promoted the accumulation of regulatory T cells (Tregs), which are immunosuppressive. SDF-1 α increased the accumulation of CXCR4⁺ tumor cells and facilitated the formation of metastatic lesions in the lymph node, as seen previously [7]. This study further confirmed the importance of this signaling pathway by using inhibitors of PGE₂, together with SDF-1 α and EP3 receptor antagonists, which inhibited lymph node metastasis in a Lewis lung carcinoma mouse model. These studies suggest that tumor-draining lymph nodes are conditioned prior to cancer cell arrival by undergoing stromal remodeling, recruiting immune cells and increasing production of chemokines. Together these alterations create a permissive microenvironment for metastases.

2.3. Lymphatic vessels: A highway for tumor metastases

A characteristic feature of rapidly growing solid tumors is an expansion of the surrounding lymphatic network. However, functional lymphatic vessels are restricted to the periphery of solid tumors [9]. Similar observations were documented in breast cancer patients [42] and advanced metastatic melanoma patients [39], where local tumor invasion correlated with high lymphatic vessel density in the tumor margin. The lack of functional lymphatic vessels inside the tumor increases interstitial fluid pressure and enhances lymph flow to the SLN [11]. A similar observation was made in experimental mouse models of cancer, where overexpression of lymphangiogenic growth factors, VEGF-C and VEGF-D, further enhanced peripheral tumor lymphatic vessel growth and increased lymph node metastasis [43–46]. VEGF-C also up-regulates CCL21 production in lymphatic endothelium, thereby promoting lymphatic entry of CCR7⁺ tumor cells [47]. Once tumor cells arrive at the lymphatic vessels, it is not yet known whether tumor cells actively or passively gain access to lymphatic capillaries.

Tumor-derived VEGF-C/D can increase contraction of proximal collecting lymphatic vessels [48], thus increasing lymph flow and potentially enhancing tumor cell dissemination. By reducing lymphatic vessel diameters, tumor spread was reduced, suggesting that tumor-induced remodeling of lymphatic vessels enhances metastasis to SLNs [11, 49, 50].

Additionally, it was shown that lymph transport in lymphatics downstream of SLN is increased and correlated with metastatic disease progression [51]. More work needs to be done to determine whether the flow patterns are altered due to the effect of VEGF-C/D on lymphatic capillaries or whether these observations are due to a direct effect on collecting lymphatic vessels. In the case of melanoma, the prevailing view is that lymph flow also promotes dissemination of cancer cells that deposit in tissues between the primary tumor and the lymph nodes, resulting in “in-transit metastases”. In later stages of tumor progression, in-transit tumor nodules were found in the collecting lymphatic vessels [52] and ultimately led to lymph node metastasis. It is generally believed that the underlying genetics of a tumor cell determines its metastatic pattern. Yet, little is known about the mechanism of how tumor cells navigate and potentially impair lymph flow, attach to lymphatic endothelium and eventually extravasate into cutaneous and subcutaneous tissue.

When lymphatic vessels are obstructed, collateral lymphatic vessels can compensate for the reduction in lymphatic capacity [14, 53, 54]. Little is known about the potential role of collateral lymphatic vessels in lymphatic metastasis. It was recently shown that lymphadenectomy of the popliteal lymph node led to alternative lymph drainage to the inguinal lymph nodes through pre-existing collateral lymphatic vessels. The drainage patterns of cancer metastasis after lymphadenectomy were rerouted, consistent with the patterns of re-directed lymphatic drainage [55]. These results call into question whether regional lymphadenectomies are sufficient to eliminate all lymph node metastases since new avenues of transport to alternative lymph nodes may be available.

2.4. Cancer cell survival in the lymphatic system

Upon entering the lymphatic vessels, cancer cells are faced with the challenge of a hypoxic environment [56]. The availability of oxygen within lymphatic vessels is influenced by tissue type, metabolic activity of lymphatic endothelium and proximity to blood vessels within the tissue. A similar challenge is faced once cancer cells reach the lymph node and enter the SCS. Under normal physiological conditions, the SCS is devoid of blood vessels and red blood cells, thus oxygen availability is scarce [57]. Cancer cells in the SCS are located 100–200 μ M from existing blood vessels within the paracortex, which is in agreement with the limits of the oxygen diffusion distance. Upon encountering a hypoxic environment, cells can either adapt to the low oxygen setting or secure an oxygen and nutrient supply by migrating to or recruiting blood vessels [58] in order to meet their energetic and biosynthetic requirements. Angiogenesis has been extensively documented to occur in response to a hypoxic environment in tumors. Production of proangiogenic factors like VEGF by hypoxic tumor and host cells can lead to angiogenesis in an attempt to eliminate local hypoxia [59, 60]. However, increases in vessel density in lymph node metastasis have not been documented to date [61–63]. Hypoxic cancer cells have the capacity to adapt in an environment that lacks sufficient levels of oxygen by up-regulating expression of glucose transporters and associated genes for anaerobic glycolysis, while local normoxic cells can utilize the resulting lactate as their energy source [64]. In addition to the effects of hypoxia on tumor cell metabolism, a chronic hypoxic environment has also been shown to select for clones that are more aggressive and metastatic [65, 66]. This selection and adaptation may enable cancer cells to survive in the SCS until invasion into the blood

vessel containing parenchyma is achieved. Alternatively, micrometastatic lesions may enter a state of dormancy while in the avascular SCS. Instead of inducing an “angiogenic switch”, tumor cells may escape this dormancy by invading the lymph node parenchyma where nodal blood vessels reside. The molecular triggers for the invasion of the parenchyma are not well understood, but chemokines might be one component [67]. One may also expect a greater degree of cellular activity and proliferation at the invasive edge of the metastatic lesion. Further investigation is warranted to fully understand the metabolic state and bioenergetic needs of tumor cells in the lymph node.

3. Chemokine signaling in lymph node metastases

After cancer cells arrive in the lymph node, further changes occur (Figure 1). Chemical signals known as chemokines help direct cells, including cancer cells, to new locations. Chemokines are a superfamily of cytokine-like molecules that can induce cytoskeletal rearrangement and directional migration of cells via their association with specific G-protein coupled receptors. Chemokines play critical roles in the migration, proliferation and maturation of immune cells in the lymph node. A growing body of literature suggests that various chemokine-receptor signaling pathways also play a central role in trafficking different cancer cell types to the lymph node. Chemokines can attract cancer cells to lymphatic vessels in the primary site, as well as attract metastatic tumor cells to secondary sites after they invade blood or lymphatic vessels. A study by Muller et al. showed that some human breast cancer cells have increased expression of several chemokine receptors including CXCR4 and CCR7 compared to normal mammary epithelial cells and this correlates with their ability to metastasize to lymph nodes [7]. Signaling through the CXCR4 and CCR7 receptors enhance actin polymerization and pseudopodia formation in breast carcinoma cells, resulting in increased motility and invasive phenotype of these cells. The ligands for these receptors are CXCL12/SDF1 α for CXCR4, and CCL19/MIP-3 β and CCL21/6CKine for CCR7, all of which are expressed in the lymph node microenvironment and can act as potent chemoattractant signals for cancer cells. To test the importance of SDF1 α /CXCR4 signaling in facilitating metastasis in a mouse model of breast cancer, anti-CXCR4 monoclonal antibody was used to neutralize its effects *in vivo*. Inhibition of SDF1 α /CXCR4 signaling significantly decreased lung and lymph node metastasis compared to isotype-treated control mice [7]. In addition to breast cancer, the importance of CCR7 signaling in mediating lymph node metastasis was elucidated in a mouse model of melanoma [68] and esophageal cancers [69]. Most recently, a role for the CCL1/CCR8, chemokine-receptor signaling pathway was demonstrated to be important for melanoma tumor cell entry into the lymph node parenchyma [67]. CCL1 is expressed by lymphatic endothelial cells in the sub-capsular sinus of the lymph node and facilitates CCR8⁺ tumor cell entry into this organ. Blocking this pathway significantly inhibited tumor cell entry into the lymph node from the afferent lymphatic vessel. Taken together, these studies show that chemokine-receptor signaling plays a crucial role in the active transport and delivery of tumor cells into the SCS and lymph node parenchyma. The importance of a specific chemokine in regulating tumor cell entry or exit from the SLN is likely dependent on the expression level of that molecule in the lymph node and the presence of its cognate receptor on cancer cells. In addition to a direct role for chemokine signaling to attract tumor cells into

the SCS of the lymph node, these molecules play important roles in immune cell chemotaxis and may be involved in establishing a pro-tumoral microenvironment in the lymph node. Thus, identifying and targeting key chemokine-receptor signaling pathways that influence tumor cell growth in the lymph node is crucial for eliminating lymph node metastases.

4. Evading immune surveillance in the lymph node

Naïve lymph nodes are the site of immune regulation where foreign, pathogenic or self-antigens accumulate. Optimal interactions between antigen-loaded-dendritic cells (DCs) and effector T lymphocytes in the lymph node facilitate adaptive immune responses that eliminate foreign and pathogenic antigens. In cancer, functional lymphatic vessels on the periphery of tumors are the physical connection between the primary tumor and SLNs. Rogue tumor cells have evolved ways of evading immune surveillance mechanisms in the lymph node and thus are able to successfully colonize and proliferate in this metastatic site. The immune microenvironment in the lymph node consists of three major cell populations, stromal, myeloid and lymphoid. Myeloid cells consist of macrophages and dendritic cells, while lymphoid cells are represented by T and B lymphocytes [70, 71]. The stromal population is comprised of fibroblast reticular cells and blood and lymphatic endothelial cells. The myeloid cells occupy only a small portion of the entire cell population in the lymph node. However, dendritic cells greatly influence immune outcome within the lymph nodes. The absence or immaturity of dendritic cells in the SLNs leads to limited T cell responses against the tumor [72, 73]. In addition, myeloid-derived suppressor cells (MDSCs) have been shown to recruit regulatory T cells (Tregs) and inhibit proliferation of T cells in SLNs [74]. Likewise, recent studies have elucidated multiple roles for stromal cells in regulating T cell fate and function in the lymph node. During chronic inflammation, activated LECs modulate the maturation of DCs and suppress their ability to induce T cell proliferation, thus preventing an immune response [75]. LECs also have the ability to delete autoreactive T cells and promote immune tolerance by constitutively expressing and presenting self-antigens on MHC class I molecules. LECs lack the co-stimulatory molecules necessary to activate CD8⁺ T cells but express high levels of the immune checkpoint inhibitory ligand PD-L1 [76]. This leads to LEC scavenging of self-antigens in the lymph fluid, followed by processing and cross-presentation of these antigens on MHC class I molecules, which then drives deletional tolerance of naïve CD8⁺ T cells [77]. This tolerance appears to be critical for the survival of tumor cells in lymph nodes since direct implantation of tumor cells into the lymph node led to rejection by a CD8⁺ T cell response [78].

Emerging evidence suggests that metastatic tumors proliferate in their new environment by evading host immune system effector functions of CD4⁺ and CD8⁺ T cells [79]. A lower percentage of effector T cells were found in metastatic and premetastatic lymph nodes [27]. Lymphocyte recruitment into the SLN is primarily via HEVs although some immune cells can also traffic into the lymph node via the draining afferent lymphatic vessels. Cancer-induced remodeling of HEVs can potentially impair immune cell trafficking to the lymph node, promoting tumor cell survival. As mentioned earlier, dendritic cell education of T cells may also be impaired, further contributing to the decrease in functional lymphocytes [72, 73]. Colorectal cancer patients with lymph node metastasis had a significant increase in CD4⁺Foxp3⁺ Tregs compared to patients with no lymph node metastasis [80]. Increased

Tregs were associated with a functional impairment of CD8⁺ T cells [80]. Among T cell populations in the lymph node, tumor-specific T cells in metastatic lymph nodes were functionally tolerant [81]. Several mechanisms have been shown to account for this tolerance including induction of tolerance by lymphatic endothelial cells [77]. Furthermore, antigen presented by tumor cells or antigen presenting cells in late-stage tumors led to proliferation of CD8⁺ T cells that lacked effector function [82].

While much attention has been paid to T cells, B cells also impact metastatic tumor growth in the lymph node [83]. B cells in the SLNs are able to process and present antigen to naïve CD4⁺ or CD8⁺ T cells, stimulating the activation of effector T cell response to tumor antigens [84, 85]. These effector CD8⁺T cells in the SLN secrete anti-tumor cytokines such as IFN- γ that mediate tumor regression. Activated B cells also have the ability to undergo clonal expansion and secrete tumor specific immunoglobulin G (IgG) in the SLN in response to tumor antigens draining to this site [86, 87]. Taken together, these studies underscore the role of B cells as efficient antigen presenting cells and regulators of T cell activation in the tumor draining lymph node.

5. Clonal dissection of lymph node metastases in solid tumors

There is much speculation about the chronological sequence of events in tumor cell metastasis from a primary location to a secondary or tertiary site. Several studies have performed lineage tracing and genomic sequencing on primary and metastatic tumors in an attempt to understand this phenomenon. A recent study characterized the clonal evolution of tumor cells in a genetically engineered mouse model of small cell lung carcinoma at metastatic sites including the lymph node and liver [88]. This model recapitulates human disease by harboring lung-specific deletion of the tumor suppressor genes, *TP53* and *RBI* and progressing from small neuro-endocrine bodies in the lung to frequent metastases in the lymph node and liver. Genomic sequencing of the primary tumor and lymph node metastases in multiple mice bearing small cell lung carcinoma revealed that tumor cells that colonized the lymph node were polyclonal as multiple primary tumor subclones were identified in the draining lymph nodes.

Another study reported a PCR-based assay to determine somatic variation in hypermutable polyguanine (poly-G) repeats as a measure of the mitotic history and clonal make-up in human cancer [89]. In a cohort of 22 patients, poly-G variants were detected in 91% of tumors and phylogenetic trees were constructed to determine the metastatic progression for each patient, most of whom were advanced colon cancer patients. This analysis revealed varying degrees of intratumor heterogeneity among patients. For example, two patients with colon cancer and distant metastases to the ovary revealed that the ovarian tumor was clonally distinct from the primary tumor and lymph node metastasis. However, two independent samples from the lymph node metastasis revealed that it had an identical genetic composition to the primary tumor, suggesting that the pool of genetically divergent clones in the primary tumor was also found in lymph node lesions. These data suggest that similar to the primary tumor, lymph node metastases represent a polyclonal population of tumor cells. This observation could have multiple implications for node positive patients. First, using targeted therapy for the treatment of lymph node metastases could be

challenging. Second, if tumor cells exit the lymph node, it is possible that multiple clones could simultaneously colonize distant sites. Finally, new driver mutations could arise in the lymph node that give rise to polyclonal distant metastases that are different from the primary tumor.

Given the polyclonality of lymph node metastases, it is unclear whether a single targeted therapy can eliminate disease. As with studies from primary tumors, lymph node metastases may develop mechanisms of acquired resistance from these therapies. The treatment strategy could be more complicated in cases where these resistance mechanisms may differ from those of the primary tumor. Clinicians and biologists are becoming increasingly aware that the mechanisms of survival and proliferation of tumor cells may be microenvironment specific, making treatment strategies complicated.

6. Lymph node metastases: Clinical perspectives

Primary tumor resection and axillary lymph node dissection (ALND) have been part of the standard treatment for breast cancer patients with metastases in the SLN. These surgeries attempt to eliminate all disease provided the cancer is in the early stages and has not metastasized to distant organs. However, ALND has several devastating short-term and long-term side effects including seromas, infections, reduced arm movement and lymphedema [90]. Due to these complications, two recent randomized clinical trials were carried out to determine if axillary dissection improves survival in early stage (I or II) breast cancer patients with a positive SLN. Both trials, the American College of Surgeons Oncology Group Z0011 trial [91] and the International Breast Cancer Study Group (IBCSG) 23-01 trial [92], showed no overall survival benefit to ALND with standard chemo-radiation therapy when compared to standard chemo-radiation therapy without axillary surgery. Similarly, the recent AMAROS (After Mapping the Axilla: Radiotherapy or Surgery?) trial showed no difference in overall survival in a randomized trial comparing ALND to radiotherapy in SLN positive patients, with the radiotherapy group experiencing less lymphedema [93, 94]. Results from these studies show that axillary dissection could be avoided in patients with early stage breast cancer and limited SLN involvement, as systemic chemotherapy or radiation therapy sterilize disease in the node. The reduced number of axillary surgeries now being performed are expected to lower the incidence of lymphedema and other complications in breast cancer patients. However, long-term follow-up studies need to be undertaken to assess whether residual disease in the node may contribute to relapse in patients who do not undergo ALND. Studies are warranted to understand the fate of cancer cells in lymph nodes of patients with early stage disease that forgo ALND as well as understanding the pathways traveled by metastatic tumor cells in patients with advanced disease involving multiple lymph nodes and distant organs. In this review series, Nathanson et. al. [5], provide a comprehensive review of our understanding of lymph node metastasis from a clinical perspective.

7. Targeting Lymph Node Metastases

Controlling the size of lymph node metastasis is important since recent clinical data have shown that breast cancer patients with micrometastases (≤ 2 mm) in their SLNs have a

reduced incidence of distant metastasis compared to those with macrometastasis [95]. Angiogenesis is required for invasive tumor growth and metastasis of the primary tumor [96]. We, and others have previously shown that antiangiogenic therapy was not as effective in stopping the initial seeding of tumor cells in the lymph node when compared to anti-lymphangiogenic therapy [11, 61, 97]. Furthermore, anti angiogenic therapy failed to inhibit growth of tumors in the lymph nodes [50], in contrast to studies where antiangiogenesis inhibitors eradicate or slow the growth of the primary tumor. Thus, treatment response in the primary tumors may not predict the response of lymph node metastases.

In patients with locally advanced breast cancer, a significant fraction of patients had residual lymph node metastases after neoadjuvant therapy, including 5-fluorouracil, doxorubicin, and cyclophosphamide, had eliminated disease in primary tumor [98, 99]. Radiotherapy has shown to be effective against such residual tumor foci in loco-regional tissue. In a meta-analysis of breast cancer patients, women with as few as 1–3 positive lymph nodes were shown to benefit from radiotherapy after mastectomy and axillary dissection [100]. However, lymph node metastases can travel beyond the sentinel and axillary lymph nodes [101]. For example, internal mammary lymph nodes (IMN) are associated with breast cancer positive axillary lymph nodes but are usually not irradiated due to cardiac toxicity. However, IMN metastases are found in at least 10% of patients where the axillary lymph nodes are negative. Overall survival and cancer-specific survival were increased in a large cohort of node negative breast cancer patients [102] that received IMN irradiation. It was found that melanoma patients with non-SLN metastases were three times more likely to die compared with patients where the metastasis was limited to the SLN [103]. Since it may be unclear as to which lymph nodes should be irradiated and whether they are accessible, such patients may benefit from therapeutic options that include radiation and a systemic chemotherapy, targeted therapy, or immunotherapy that will eradicate occult cancer cells in all lymph nodes.

Several reports have shown that subcutaneous chemotherapy targeting regional lymphatic tissues provides greater drug concentration in the lymph node compared to intravenous delivery [104]. It is unknown whether subcutaneous administration will lead to lower rates of residual tumor in the lymph node. However, by intravenous route, drug delivery to the lymph nodes of HIV⁺ patients has been shown to achieve lower concentrations than that found in the blood and other organs, which correlated with increased viral replication in the lymph node compared to blood [105]. Recent approaches have focused on improving drug exposure, retention, and distribution within the lymphatic system. Using lipid nanoparticles, the concentration of anti-retroviral drug was significantly increased in lymph nodes throughout a primate compared to standard oral formulations [106]. Such approaches will likely also enhance the targeting of tumor cells in the lymph node.

There is therapeutic potential in reversing tumor-induced immune suppression by targeting “metastatic niche” molecules. This strategy may prevent the spread of cancer to distant lymph nodes and/or organs. Taking advantage of the tumor antigens that are present in SLNs, Thomas et al. used nanoparticles to deliver adjuvant to SLN to enhance immune activation. This approach led to decreased tumor growth by altering the dendritic cell profile and increasing Th1 immunity within the tumor [107]. GM-CSF, a multifunctional cytokine,

is necessary to induce expansion of a population of myeloid-derived suppressor cells that lead to tolerance in secondary lymphoid organs. Blocking GM-CSF reduced tumor-induced tolerance by enhancing tumor specific, IFN- γ -producing CD8⁺ lymphocytes in lymph nodes [33]. Likewise, inhibition of TGF- β from the primary tumor decreased the number of regulatory T cells and increased the number of tumor antigen-specific helper and cytotoxic T cells that produced IFN- γ [108]. This approach led to diminished growth of distant metastases. Further approaches to stimulate the immune system to target lymph node metastasis are under development.

8. Conclusions and Future Perspectives

Studies focused on understanding the process of lymphatic metastases and the role of the lymph node in tumor progression in the past decade have given us insights into the importance of these organs in cancer biology. Metastasis to the lymph node is preceded by changes in the lymph node microenvironment. A more comprehensive understanding of the molecular signals in the premetastatic lymph node niche that facilitate tumor metastasis could provide therapeutic targets to control lymph node metastasis. Although several advances have been made in the field of lymphatic metastasis, unanswered questions regarding the effects of the lymph node microenvironment on the metastatic potential of tumor cells in the lymph node or on anti-tumor immune response still remain. The ability of metastatic tumor cells in the lymph node to progress to distant sites remains controversial and we lack biomarkers to identify patients at risk of systemic spread from lymph node metastases. With continued research progress to address these issues, we will improve treatment and outcomes for patients with lymphatic metastasis.

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Abbreviations

ALND	Axillary Lymph Node Dissection
BMP-4	bone morphogenetic protein-4
DC	Dendritic cell
EPO	erythropoietin
HEVs	high endothelial venules
IFN-γ	interferon γ
IL-10	interleukin-10
IMN	internal mammary lymph nodes
LEC	Lymphatic endothelial cell

LYVE-1	lymphatic vessel endothelial hyaluronan receptor
MDSC	myeloid-derived suppressor cells, PGE ₂ , prostaglandin E ₂
PD-L1	programmed death-ligand 1
PNAd	peripheral node addressin
SDF-1α	stromal derived factor-1 α
SCS	sub-capsular sinus
SLN	Sentinel Lymph Node
TGF-β	transforming growth factor β
Treg	regulatory T cells
VCAM-1	vascular cell adhesion molecule 1
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor

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Highlights

- Lymphatic endothelial cells and vessels actively participate in tumor cell metastasis
- The premetastatic lymph node expresses immunosuppressive signals that condition this organ for the arrival of tumor cells
- The composition of a metastatic lymph node undergoes remodeling that impacts the growth of cancer cells
- Tumor lesions in the lymph node are polyclonal and mostly represent the genetic composition of the primary tumor
- The ability of tumor cells residing in the lymph node to colonize distant organs is controversial

Figure 1.

Alterations to tumor draining lymph nodes during cancer progression. A) Naïve lymph nodes are sites where foreign, pathogenic or self-antigens accumulate and adaptive immune responses are generated. Optimal interactions between antigen-loaded-dendritic cells and effector T lymphocytes in the lymph node stimulate effective immune responses that eliminate foreign and pathogenic antigens. B) Prior to cancer cell dissemination, tumor draining lymph nodes undergo many remodeling processes, including lymph node lymphangiogenesis, alterations in high endothelial venules, increases in chemokine and cytokine production and alterations in immune cell composition. These changes are thought to enable the growth of cancer cells that are seeded in the lymph node. C) Cancer bearing lymph nodes, undergo further changes, including the loss of high endothelial venules, regression of lymphangiogenic vessels, and impairment of lymphocyte recruitment. These changes inhibit adaptive immune responses and may enable the further spread of cancer cells to distant organs.