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Lymphatic Vessels, Inflammation, and Immunity in Skin Cancer

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Abstract

Skin is a highly ordered immune organ that coordinates rapid responses to external insult while maintaining self-tolerance. In healthy tissue, lymphatic vessels drain fluid and coordinate local immune responses; however, environmental factors induce lymphatic vessel dysfunction, leading to lymph stasis and perturbed regional immunity. These same environmental factors drive the formation of local malignancies, which are also influenced by local inflammation. Herein, we discuss clinical and experimental evidence supporting the tenet that lymphatic vessels participate in regulation of cutaneous inflammation and immunity, are important contributors to malignancy and potential biomarkers and targets for immunotherapy.

Keywords

skin cancer; lymphangiogenesis; immune microenvironment; inflammation

Skin is the largest organ in mammals, serving as a physical and immunological barrier to the external environment (1). As an immune organ, skin coordinates rapid responses to external challenge through constitutive immune surveillance involving both resident and recruited leukocytes (2). Exquisite spatiotemporal regulation of immune cells is required for homeostatic tissue maintenance - loss of function is associated with a variety of

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dermatopathologies including chronic infection, inflammation, autoimmunity and cancer (1). The interplay between local inflammation, tissue remodeling and anti-tumor immune responses are of particular interest given recent success of immunotherapies, namely blockade of immune checkpoint molecules (3). Since tumor microenvironments in part regulate anti-tumor immunity, these also present targets for release of local immunosuppression that may synergize with checkpoint therapies (4). While tumor-associated blood vessels are appreciated for their role in regulating leukocyte infiltration and function (5), tumor-associated lymphangiogenesis has lagged behind with respect to recognition of its role(s) tumor-associated inflammation and immunity. Recent revelations, however, have highlighted these important roles with respect to coordination of local inflammation (6) and immunity (7–11), indicating that their deregulation may similarly influence inflammation-induced tumor progression and metastasis.

In this review, we discuss clinical and experimental evidence revealing the significant inflammatory and immunomodulatory functions of lymphatic vasculature, and suggest how tumor-associated remodeling may alter regional inflammation and immunity, thereby contributing to disease progression and metastasis. In addition, we suggest that lymphatic vessel morphology and function may be a biomarker of cutaneous immune status, and discuss how lymphatic biomarkers may provide insight into diagnosis and therapy for cutaneous malignancy.

Inflammation and Cancer

It is now well appreciated that environmental exposures account for occurrence of many different types of cancer, with 15–20% of cancer deaths can be linked to inflammation or infection (12). Environmental factors include infectious agents, physical trauma (non-healing scars, burns), industrial toxins (e.g. tar, arsenic, radiotherapy), as well as those associated with lifestyle (tobacco, alcohol, diet, ultraviolet exposure) (13). As one might expect, tumors whose etiology is associated with environment-induced inflammation often occur in tissues with greatest surface area including lung, skin and gastrointestinal tract (14). Inflammation is considered one of ten hallmarks of cancer and is increasingly appreciated for its complex role in tumor initiation, promotion, malignant conversion and metastasis (15). Furthermore, immune infiltrates provide prognostic biomarkers (16) and potent targets for cancer therapy (17). We are just beginning to understand, however, how complex interactions between environmental agents, genetic factors and tissue microenvironments coordinate local inflammation and subsequent tumorigenesis.

Skin Inflammation and Cancer

Skin is a stratified tissue composed of epidermal, dermal, and subcutaneous adipose layers functioning in concert as a barrier to the external environment (1). Keratinocytes are the primary cell type in epidermis and undergo continuous cycles of homeostatic proliferation and differentiation to maintain a cornified layer protecting against water loss and microbe penetration (18). Melanocytes reside in basal layers of epidermis. Dermis is largely composed of collagenous and non-collagenous (e.g. elastic fibers and structural proteoglycans) connective tissue components that provide substratum support for blood and

lymphatic vessels, nerves, mesenchymal support cells (fibroblasts), and resident immune cells. The cellular components of these layers critically regulate immune surveillance to facilitate rapid responses to insult while importantly maintaining immune tolerance (2,19) - breakdown of this balance is associated with a diverse set of immune-mediated dermatopathologies (1).

There are four major types of skin cancer, basal cell carcinoma, squamous cell carcinoma, melanoma and non-epithelial skin cancers (e.g. Merkel Cell, Kaposi's sarcoma and cutaneous lymphoma). Predisposition to skin cancer is associated with chronic cutaneous inflammation (e.g. discoid lupus erythematosus, chronic wounds, dystrophic epidermolysis bullosa), viral infections (e.g. human immunodeficiency virus, human herpes virus 8 and human papilloma virus) and inflammatory environmental agents (e.g. UVR, viral infection, aging, diet, smoking, radiotherapy, phototherapy, physical trauma) (13), with inflammation playing a well-documented role in regulating progression of these cutaneous malignancies (20), however, skin cancers are also associated with chronically injured or non-healing scars, burns and chronic friction, with incidence of malignancy in scar tissue being low (0.1–2.5%) (13).

Furthermore, cells in skin secrete a diversity of inflammatory mediators, including toll-like receptor (TLR) ligands, polypeptide growth factors e.g. tissue necrosis factor (TNF) α , as well as chemokines that direct trafficking and subsequent activation of recruited leukocytes; many of which have been implicated as mediators of tumor progression in skin (20). For example, mast cells foster skin carcinogenesis through release of pro-survival and pro-angiogenic polypeptide growth factors and matrix remodeling proteinases that activate proliferative programs in keratinocytes, fibroblasts and endothelial cells (21). B cells secrete (auto)antibodies that form immune complexes with complement proteins – these accumulate in skin and influence resident and recruited myeloid cells activation and function (20). Tumor-infiltrating leukocytes play an important role in regulating angiogenic responses, which in turn, regulate their entry (22), and thereby influence metastatic potential (23). While much focus has been placed on blood vasculature with respect to leukocyte infiltration and function, lymphatic endothelium may similarly participate in these complex interactions. In support of this, using a murine model of chemically-induced skin carcinogenesis, it was recently reported that lymphatic vessels and their subsequent drainage are required for induction of local leukocytic infiltration, and consequently their absence resulted in initiation of fewer tumors (6). Given our current understanding of how lymphatic vessels participate in regulating local inflammation and immunity, as described below, it is reasonable to hypothesize that tumor-associated lymphatic remodeling participates in shaping immune microenvironments of solid tumors.

Lymphatic Vessels and Regional Inflammation

Lymphatic vessel architecture dictates local flow and the unidirectional transport of peripheral information to the secondary lymphoid system (24). Lymphatic vessels develop as a hierarchical, one-way drainage system functioning to transport cells and fluid into the lymphoid system and as a result of this architecture, lymphatic vessels lie at the interface of tissue biology and immunology (24). In skin, lymphatic vessels form two plexuses, one

superficial extending into dermal papillae, and the other near the subpapillary arterial network (Figure 1A). Initial lymphatic capillaries are small, blind-ended vessels with minimal basement membrane that directly attach to extracellular matrix (ECM) through anchoring filaments allowing for rapid response to changes in local interstitial fluid pressure; swelling of ECM due to edema pulls on these filaments opening the loose, button-like inter-endothelial junctions (Figure 1B) (25–28).

Lymphatic capillaries drain into collecting vessels characterized by a continuous basement membrane, smooth muscle coverage and a system of valves that prevent retrograde fluid flow (Figure 1C). Lymphatic dysfunction results in accumulation of protein-rich interstitial fluid (lymphedema) leading to progressive fibrosis, adipose deposition and inflammation (29). While lymphatic vessels are required for drainage of soluble antigen and trafficking of activated dendritic cells (DCs) to local lymph nodes, their contribution to other aspects of inflammation and immunity are just being explored. There have been several excellent reviews highlighting current knowledge of lymphatic vessels to inflammation (30) and tolerance (31). Herein, we highlight their roles in immune induction and resolution or tolerance (Figure 2A–D), and discuss how their dysfunction contributes to tumor progression and metastasis. Of note, as many of these studies were not performed in skin, it remains to be seen how many of these mechanisms will translate across tissue systems.

Inflammation and Immune Induction

Lymphatic vessels in adult tissues are typically quiescent, but may become activated when inflamed, resulting in lymphatic vessel remodeling within peripheral tissue and draining lymph nodes (26). Lymphatic remodeling is defined as changes to lymphatic vessel structure and morphology either through mechanisms of proliferative lymphangiogenesis or lymphatic vessel enlargement (26). Lymphangiogenesis specifically describes the formation of new lymphatic vessels from pre-existing vessels and occurs in many experimental and clinical inflammatory diseases including transplant rejection (renal and cornea), inflammatory bowel disease, chronic airway inflammation, and psoriasis (26,32–35). Though associated with various inflammatory settings, it remains unclear as to whether lymphangiogenesis is a driving force in generation of inflammation and pathology or instead is an active attempt to resolve the inflammatory process.

Vascular endothelial growth factors (VEGFs) and their receptors are largely responsible for development, sprouting and remodeling of lymphatic vessels (26). Both hematopoietic and non-hematopoietic cells in skin orchestrate lymphangiogenic responses during inflammation. Epithelial cells in skin and fibroblastic reticular cells in lymph nodes support VEGF-A-dependent lymphangiogenesis (34,36) while macrophages induce local lymphangiogenesis largely through secretion of VEGF-C, that in turn signals through vascular endothelial growth factor receptor (VEGFR) 3, also required for developmental lymphatic vessel growth (37–39). VEGF-A producing B cells are required for inflammatory lymphangiogenesis in draining lymph node (40), but can be compensated for by neutrophils, likely through modulation of VEGF-A bioavailability and some VEGF-D production (41). In contrast, T cells can inhibit *de novo* lymphangiogenesis in an interferon (IFN)- γ -dependent manner at least in lymph nodes (42). Importantly, at least in the respiratory tract,

inflammatory lymphangiogenesis is characterized by remodeling of the loose, button-like interendothelial junctions into tight, zipper-like junctions, characterized by firm adhesion along the length of two neighboring cells (Figure 1A) that may have functional consequences for mechanisms of immune cell entry and fluid transport (25,28).

Mobilization of DCs towards draining afferent lymphatic vessels is dependent on expression of the C-C chemokine receptor 7 (CCR7) that allows for active homing towards the C-C motif ligand 21 (CCL21) and CCL19-producing lymphatic vasculature (43). In addition to CCL21/CCL19, other chemokine signals required for cell entry into afferent lymph include chemokine C-X-C motif chemokine ligand (CXCL)12, sphingosine-1-phosphate (S1P), CX3CL1, the decoy receptor D6 (44). Also involved are adhesion molecules including intracellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), L1CAM (CD171), ALCAM (CD166), C-type lectin receptor (CLEC-2), CD31, semaphorins, CD73, and the scavenger receptor CLEVER-1 (44,45). Whether integrins are absolutely required for DC transmigration is debated but is likely a function of inflammatory context and lymphatic endothelial cell (LEC) activation status (45–49). Cytokines, toll-like receptor (TLR) ligands and interstitial fluid flows all alter expression of adhesion molecules on LECs so as to promote leukocyte migration (44), and LECs derived from various inflammatory contexts acquire distinct transcriptional programs (50) indicating that LEC function and leukocyte trafficking patterns may be inflammation-specific. Furthermore, the endothelial glycoprotein PLVAP expressed by LECs controls entry of both lymphocytes and antigens into lymph nodes (51). The PLVAP protein acts as a diaphragm spanning transendothelial channels that transect sinus-lining LECs and confer selectivity of the sinus-parenchyma barrier (51). This selectivity may provide a way for the host to segregate small, likely inert proteins, which enter the lymph node conduit system (52) from larger agents that might cause damage if disseminated systemically.

One class of proteins that regulate or tune innate and adaptive immunity are the chemokine decoy receptors that scavenge and sequester chemokines to decrease local inflammatory signaling (53). This subfamily of “silent” chemokine receptors includes Duffy antigen receptor for chemokines (DARC), D6 (also known as CCBP2), and CCX-CKR (also known as CCRL1) and are strategically expressed in distinct cellular contexts (e.g. DCs and endothelial cells) where they regulate spatiotemporal inflammatory β -chemokine signaling (53). D6 is predominantly expressed by LECs (skin, gut, lung and syncytiotrophoblast layer of the placenta) - D6-null mice are unable to properly resolve local acute inflammation following dermal challenge due to exaggerated cutaneous inflammatory responses characterized by an accumulation of β -inflammatory chemokines at sites of inflammation (54), a process that may prevent further leukocyte recruitment. Furthermore, D6, induced during inflammation by interleukin-6 and IFN γ , facilitates selective presentation of homeostatic chemokines (e.g. CCL21) over inflammatory chemokines to prevent inappropriate inflammatory cell attachment to LECs and proper selection of mature over immature DCs (55). Extensive perilymphatic accumulation of leukocytes is observed in D6-null mice in both peripheral sites of inflammation and draining LNs, resulting in lymphatic congestion and impaired transport of antigen presenting cells and fluid (56). Importantly, D6 is downregulated in several human malignancies including Kaposi sarcoma, a cutaneous

malignance of lymphatic endothelial origin, where low levels of D6 is associated with disease aggressiveness and infiltration of proangiogenic macrophages (57)

Lymphatic control of dendritic cell trafficking through both adhesion molecules and chemokines is an finely-regulated, multi-step process (45,49). Furthermore, given the intimate relationship peripheral afferent lymphatic vessels have with egressing leukocytes (45), it remains plausible that egressing leukocytes are educated by afferent lymphatic vessels as they exit peripheral tissue. The characterization of specialized three-dimensional structures at the interface of DC/LEC adhesions (58), intralymphatic crawling of DCs following entry into peripheral initial capillaries (59), acquisition of peptide:MHCII complexes by LECs from DCs (60) and LEC-mediated inhibition of DC maturation (61) all suggest that long-lived intercellular interactions between egressing leukocytes and peripheral afferent lymphatic vessels may be a novel control point for leukocyte function.

Tissue Egress and Immune Resolution

In addition to providing an initial route for entry of newly activated DCs into the afferent lymph, lymphatic vessels may also provide an important route of egress during resolution of acute and chronic inflammation. Consistent with this idea, induction of lymphatic vessel growth in skin is protective against acute and chronic skin inflammation (62,63), and inhibition of vessel growth in experimental inflammatory bowel disease exacerbates inflammation and disease progression (35). In murine inflammatory bowel disease macrophage egress from tissue in response to VEGF-C driven lymphangiogenesis is critical for limiting local inflammation (35). Similarly, in a mouse model of chronic respiratory tract infection, airway inflammation promoted bronchial lymphedema and airway obstruction when lymphangiogenesis was impaired (64). Furthermore, homeostatic gut leukocytes broadly migrate to draining LNs (65), whereas during cutaneous immunity regulatory T cells preferentially egress from skin (66).

Taken all together, dynamic patterns of leukocyte infiltration and egress appear to contribute to disease and control over leukocyte trafficking and tissue egress may be a novel point of immune regulation (67). Such a strategy is utilized by viruses to mediate immune escape (67). IFN α production following viral infection (68) and local skin irradiation (69) drive accumulation of cells in lymphoid organs, resulting in decreased peripheral blood and thoracic duct lymphocytes and overall immune suppression. Egress from peripheral tissues appears tightly controlled by a combination of CCR7 and sphingosine-1-phosphate (S1P) signaling, though coordination of these signals is poorly understood. In models of lung and skin infection, T cell egress from acutely inflamed peripheral tissue is dependent on CCL21/CCR7 chemotactic signals (70,71), while chronic inflammation adopts CCR7-independent mechanisms for tissue exit (72). Regulated tissue exit is likely required for recirculation of non-antigen specific T cells recruited sites of inflammation. Consistent with this, an interplay exists between antigen recognition and egress, where T cell receptor (TCR) stimulation results in downregulation of CCR7 and tissue retention (67). In solid tumors, at least in some cases, where antigen recognition can be limited due to physical constraints of tumor microenvironments as well as lack of potent neo-epitopes for TCR-recognition (73), cellular egress from tissues may correlate with poor local immunogenicity.

S1P gradients also importantly regulate tissue entry and exit throughout the body (e.g. blood to lymphoid tissue to lymph to peripheral tissue) (74). Lymphatic vessels express sphingosine kinase and maintain high levels of S1P in lymph generating gradients to direct tissue egress at steady state (75). Increased levels of S1P are present in inflamed peripheral tissues and this high tissue concentration may play a role in T cell retention (76). The S1P receptor, S1P₁, inhibits migration of T lymphocytes into afferent lymphatic vessels both at steady state and during inflammation, resulting in their retention in non-lymphoid tissues at least partially through LFA-1/ICAM-1 and VLA-4/VCAM-1 interactions (76). The interplay between these signals provides a novel mechanism of control over leukocyte accumulation within peripheral tissue and subsequent disease progression. All together, these mechanisms suggest that lymphatic vessel function influences accumulation and/or retention of leukocytes within inflamed tissue thereby actively contributing to disease progression.

Tolerance

While the role of the lymphatic vasculature in cellular trafficking is appreciated, novel functions of lymphatic vessels and endothelial cells comprising their structure are emerging and indicate a role for lymphatic vessels in local immunomodulation and tolerance (24). LECs inhibit T cell activation and expansion through both antigen-dependent (7–10) and independent (77) mechanisms. While central mechanisms of tolerance eliminate self-reactive T cell clones during development in the thymus, education of the immune system is incomplete. As a consequence, tolerance must be maintained in the periphery to both environmental and self-antigens to prevent allergy and autoimmunity, respectively. Reminiscent of thymic epithelium, which deletes auto-reactive T cells in the thymus, LECs express multiple peripheral tissue antigens and directly present to auto-reactive T cells, albeit by a mechanism independent of the autoimmune regulator Aire (9). Direct presentation of the melanocyte-specific protein tyrosinase on major histocompatibility complex I (MHC I) by lymph node LECs mediates deletion of tyrosinase-specific T cells (10). T cell tolerance, in this context, is mediated by expression of the immune checkpoint molecule programmed death ligand 1 (PD-L1) on LECs and lack of costimulatory molecules; loss of PD-L1 activates tyrosinase-specific CD8⁺ T cells responses resulting in experimental vitiligo (10). While hyper-activation of melanocyte-specific T cells results in autoimmunity, vitiligo is prognostic for effective immunity during spontaneous regression of melanoma and following immunotherapy in melanoma patients (78). Induction of anti-self responses, in either an autoimmune or neoplastic context, requires a break in natural mechanisms of peripheral tolerance in which lymphatic vessels and their expression of PD-L1 play at least some role. Overexpression of PD-L1 is a hallmark of adaptive immune resistance in melanoma and a potential barrier to immunotherapy (79). While expression of PD-L1 is largely attributed to tumor cells, a wide variety of stromal cells also express PD-L1, and it remains to be determined how these players may contribute to immune suppression in tumor microenvironments (17).

In addition to direct presentation of peripheral antigens, LECs are phagocytic, scavenge lymph-borne antigen and cross-present to MHC I in a transport associated with antigen processing 1 (Tap1)-dependent manner (7,8). Again, presentation in this context results in lack of costimulation and dysfunctional T cell activation characterized by reduced IFN γ

production, high-levels of PD-1 expression and enhanced apoptosis (7,8). Furthermore, overexpression of VEGF-C in murine melanomas results in immune protection against induced immunity, and specific cross-presentation of exogenous, tumor-associated antigens (ovalbumin) (7). In both the tumor context and at steady state, presentation of the CD8 epitope for ovalbumin by tumor-educated LECs results in dysfunctional activation of antigen-specific CD8 T cells (7,8). Interestingly, phagocytic activity of lymph node-resident LECs has recently been reported in a vaccine model, where LECs were long-term depots for vaccine antigen (80). While in these studies the authors did not demonstrate cross-presentation of antigen by LECs, this antigen-capture and archiving provided enhanced protection against infection (80). Understanding the contextual cues driving LEC decision making with respect to antigen capture and presentation will be of undeniable importance moving forward with respect to vaccination strategies and understanding the role of persisting and chronic antigen exposure during vaccination, infection and tumorigenesis.

Environmental Agents and Lymphatic Function

Under homeostatic conditions, lymphatic vessels facilitate acute immune induction followed by appropriate resolution as described above. However, environmental risk factors for malignancy (ultraviolet radiation, infection, and sustained trauma) also induce lymphatic vessel dysfunction and may disturb this critical immune balance in tissue (Figure 3). In this way, regions of skin can acquire sectorial immune dysfunction, e.g. immune compromised districts, over time due to accumulation of environmental insults even in the absence of systemic immune disorder (81). These ‘districts’ exhibit enhanced risk for recapitulation of cutaneous disorders specifically at sites of trauma or insult (e.g. herpetic infections, irradiation, and burns) (81), or instead can conversely be spared from systemic immune pathology (81), and thus establishing regional discrepancies in immune function. How localized immune dysfunction may contribute directly to malignancy remains unclear, however, the coordinated dysfunction induced by these agents on both cells that acquire tumorigenic capacity and surrounding tissue stroma (including lymphatic vessels) is interesting to consider.

Ultraviolet radiation (UVR) is the most significant environmental risk factor for development of cutaneous malignancies (13). In addition to direct roles in initiating oncogenic mutations, UVR drives leukocyte infiltration of tissues, suppresses local T cell function, and induces lymphatic vessel dysfunction (82). Clinically, lymphatic vessels are reduced in number and dilated in sun-damaged skin as compared to non-sun exposed areas (83), and pathologically-dilated lymphatic vessels are associated with severe photoaging (84). Similarly, in mice, acute exposure to UVB radiation induces leukocyte infiltration of skin associated with epidermal hyperplasia, erythema and increased lymphatic vessel leakiness leading to edema (85,86). Maintenance of lymphatic vessel integrity following UVR is at least in part due to tight junction molecules (claudin-5 and ZO-1) and administration of angiopoietin 1 (Ang1) improves integrity (85,87). Disruption of claudin-5 junctions exacerbates not only edema associated with UVB exposure, but also accompanying leukocytic infiltrate (85), while overexpression of Ang1 is instead protective (87). Overexpression of VEGF-C attenuates UVB-induced edema and leukocytic infiltrate through the promotion of local lymphangiogenesis (63).

A second environmental influence on lymphatic vessels is infection. Recurrent herpetic infections are a clinical cause of secondary lymphedema and are often associated with localized immune dysfunction (88). The majority of experimental data exploring neovascularization responses to viral infection in mice have focused on the cornea, a normally alymphatic tissue, where local induction of lymphangiogenesis is associated with wounding, graft rejection and loss of immune privilege (32). In particular, herpes simplex virus 1 (HSV-1) infection induces genesis of lymphatic vessels into cornea in a TLR-independent, VEGF-A-dependent manner (89). In this model lymphangiogenesis precedes angiogenesis and is maintained at later time points by VEGF-C produced by local T cells (90). In addition to herpetic infections, skin infection by human papilloma virus (HPV) similarly results in microscopic regions of lymphatic dysfunction marked by increased number and severe dilation of local vessels (29). These regions are also associated with immune dysfunction and emergence of dermatopathology (clinical warts) associated with latent HPV infection following local trauma (29). HPV16-associated cancers exhibit lymphangiogenesis as well, and both remodeling of lymphatic vessels and expression of VEGF-C are associated with increased risk and lymph node metastasis (91). In humans, lymphatic vessel density in squamous cell carcinomas is increased immediately adjacent to tumor nests (91). Similarly, in a model of murine *de novo* squamous cell carcinogenesis driven by HPV16 oncoproteins, pre-malignant lymphatic vessels are structurally altered but functional, and progressively lose drainage function in central regions of tumors resulting in disruption of tissue hemodynamics (92).

Finally, surgical or other physical trauma result in disruption of regional lymphatic vessel networks (e.g. following radical mastectomy and lymph node resection) thereby leading to local lymphedema. The frequency of extremity (arm) lymphedema following surgical intervention for breast cancer ranges from 21% to 40% depending on both the type of surgery, and use of adjuvant radiotherapy (29). Localized lymphedema in scar and adjacent tissues has been observed, and high incidence of bacterial and fungal infections at sites of amputation is indicative of impaired local immunity, with stump skin found to exhibit an altered microbiome as compared to a non-amputated regions (88). The incidence of malignancy in scar tissue is 0.1–2.5% (13), however, the drivers of malignant conversion in these injured tissues and what role if any lymphatic function may play remains unknown. Interestingly, VEGF-C therapy may restore lymphatic function at least in some contexts to prevent surgery-associated lymphedema. Administration of growth factors along with lymph node transfer in pigs promoted robust growth of lymphatic vessels helping to preserve transferred node structure and normalize lymphatic vascular anatomy (93).

Lymphatic Vessels, Inflammation and Metastasis

Lymphatic vessels have been evaluated in the context of many solid tumors for their ability to predict locoregional, lymph node metastasis and overall survival (94). We limit our discussion here to the interplay between inflammation and tumor-associated lymphangiogenesis and direct the reader to recent reviews that cover mechanisms of lymphangiogenesis, lymphogenous tumor spread and seeding in more detail (26,27). The major molecular drivers of tumor-associated lymphangiogenesis are VEGF-C and -D, produced both by tumor and infiltrating myeloid cells (26). VEGF-C and -D exert their

biological effects through binding to VEGFR-3 and VEGFR-2, leading to activation of receptor-tyrosine kinase activity through auto-phosphorylation, which in turn activates aspects of LEC function, and vessel formation (95,96). VEGF-C induces endothelial cell migration, permeability, proliferation, vessel enlargement and enhanced trafficking of neoplastic cells to lymph nodes, largely via VEGFR-3-dependent mechanisms (96–100). VEGFR-3 expression correlates with lymphatic metastasis in some human tumors and high VEGF-C and -D expression is associated with increased lymphatic vessel density and lymphovascular invasion in both human tumors and animal models (97,99–102), and consequently inhibition of either VEGFR-3 or its coreceptor neuropilin 2 reduces incidence of lymph node metastasis (97,103–105).

In skin cancer the overwhelming majority of lymphatic vessel biomarker studies have focused on metastatic melanoma (94), where increased lymphatic vessel density correlates with VEGF-C expression in tumor microenvironments, lymphatic vessel invasion and lymph node metastasis (39,106,107). While several studies have demonstrated a positive correlation between lymphatic markers and survival (26,106) others have failed to demonstrate a correlation (108,109), thus generating some controversy over the true independent prognostic value of lymphatic vessel density and invasion biomarkers. In addition to melanoma however, recent studies have reported increased lymphangiogenesis in dermis adjacent to tumor nests in squamous cell carcinoma that also exhibit enhanced levels of VEGF-C produced by CD163⁺CD68⁺ macrophages (91). Similarly, an increase in the absolute numbers of lymphatic capillaries was observed in Merkel cell carcinoma, likely driven again by CD68⁺CD163⁺ tumor-associated macrophages (110), and increased levels of VEGFR3⁺ cells (both tumor and stroma) and lymphatic (podoplanin⁺) vessels correlated with disease progression in one study of Sézary syndrome, an aggressive subtype of cutaneous T-cell lymphoma (111).

In addition to remodeling of vessels in the primary tumor, significant enlargement of lymph sinuses in tumor-reactive lymph nodes is observed before arrival of metastatic cells (112). Distal remodeling of lymphatic vessels, both structural and functional, is mediated at least in part by VEGF-C and neuropilin-2 (98,113,114) and these changes may play a role in establishing pre-metastatic niches for locoregional dissemination. Importantly, lymphatic remodeling induced by these signals is also associated with altered fluid flows that in turn correlates with lymph node metastasis (97–100,115). Dynamic lymphoscintigraphy that traces accumulation of radionuclide over time in draining LNs reveals that increased rates of lymphatic flow associate with increased incidence of metastasis and lymphatic vessel density in primary tumors (116). Moreover, VEGF-C overexpression further promotes lymph flow (116), and exogenous application of VEGF-C reduces tumor interstitial fluid pressures (117).

It was previously thought that functional lymphatic vessels were excluded from solid tumor parenchyma, thus negating their role in tumor progression. However, neither intratumoral lymphangiogenesis nor intratumoral lymphatic vessels are required in all cases for lymph node metastasis (118,119). Furthermore, an impaired capacity of lymphatic vessels to transport fluid does not necessarily correlate with their role in transporting cells, and instead, remodeling of peritumoral lymphatic vessels and activation of existing vessels is sufficient

for altered homing properties and metastatic potential (98,118,119). The mechanisms by which lymphatic vessels actively recruit and promote seeding of tumor cells has been reviewed elsewhere (26). It is of note, however, that direct evidence for a sequential model of metastasis whereby lymphogenous spread precedes hematogenous colonization of distal organs is lacking (26,120); thus the relative contribution of tumor-associated lymphatic remodeling and metastasis to overall survival and disease progression remains somewhat unclear.

What may contribute to the association of lymphatic remodeling with poor prognosis is its relationship with local inflammation. Clinical and histopathological studies have demonstrated a correlation between cyclooxygenase (COX)-2 expression, lymphatic vessel density and lymph node metastasis in human malignancy (121–124). Current data indicates that the prostaglandin pathway influences lymphangiogenesis through upregulation of VEGF-C and -D expression in tumor-associated macrophages (125), as well as in neoplastic cells (126); however, the role of prostaglandins in lymphangiogenesis is not tumor-specific. In a subcutaneous matrigel plug assay of granuloma formation, lymphangiogenesis was also COX-2 dependent, requiring the prostaglandin E receptor (EP) 3 and 4 and macrophage production of VEGF-C (125). Similarly, COX-2 in the microenvironment of involuting mammary glands contributes to normal involution-associated lymphangiogenesis as well as mammary tumor-associated lymphangiogenesis, tumor cell invasion into lymphatic vessels and distal metastasis (127). Interestingly, it was recently reported that LECs regulate prostaglandin degradation downstream of VEGF-D/VEGFR-3/2 signaling resulting in the accumulation of tissue PGE₂ and dilation of collecting lymphatic vessels draining the tumor (128). This dilation is associated with VEGF-D expression and enhanced LN metastasis in both human and mouse models (128). Specifically in skin, UVR stimulates COX-2 expression in epidermis (129) - the EP1, 2 and 4 receptors have been linked to UV-induced carcinogenesis (130), and notably inhibition of COX-2 via administration of celecoxib, prevents both squamous and basal cell carcinoma development in mice and humans (131,132).

Therapeutic Implications

Targeting Lymphangiogenesis

Targeting of tumor-associated lymphangiogenesis as a potential therapy for solid tumors has been attempted in both pre-clinical and clinical trials (27,94). It is difficult, however, to separate effects on the lymphatic vasculature from hematogenous vessels given overlap in mechanisms required for their remodeling and growth, namely VEGFR3 (104,133,134). Either way, direct targeting of tumor vasculature in the clinic with FDA-approved drugs such as bevacizumab, sorafenib and sunitinib that target VEGF-A and kinase activity of VEGF family receptors (all of which may also interfere with signaling on tumor-associated lymphatic endothelium) provide only transient benefit and ultimately tumors adapt and regrow (133).

Alternatively, it has been suggested that indirect inhibition of vascular remodeling may be induced through repolarization of immune microenvironments (135). Numerous studies have revealed that recruitment of myeloid cells facilitates the angiogenic switch through

expression of VEGF and liberation of pro-angiogenic molecules from ECM (20,21). Targeting recruitment of these cells into tissue parenchyma results not only in release of local immunosuppression (i.e. increased effector T cell activity), but also a simultaneous normalization of angiogenic vasculature (135). Given the role local inflammation also plays in lymphangiogenesis it is reasonable to speculate that these strategies may similarly affect lymphatic vasculature. Use of non-steroidal anti-inflammatories (NSAIDs) for chemoprevention of cancer support this claim, as discussed above, where COX-2-dependent tumor formation is also associated with lymphangiogenesis in tumor microenvironments and LN metastasis (128,136,137).

Consequently, prophylactic treatment with inhibitors of COX-1 and 2 may halt tumor progression through simultaneous inhibition of local inflammation, lymphangiogenesis and angiogenesis (Figure 4A and B) (130,137). One caveat to direct or indirect anti-lymphangiogenic therapy, however, is evidence indicating that lymphatic vessels are resistant to 'normalization' therapy. Experimental evidence for this is provided by studies of *mycoplasma pulmonis* infection of murine respiratory tracts where angiogenesis and lymphangiogenesis are associated with infiltrating leukocytes (34). Resolution of inflammation through dexamethasone administration resolved the angiogenic responses but failed to resolve changes in lymphatic vessels, indicating that aberrant lymphatic vessel responses are long-lived and do not require continued maintenance. Only when dexamethasone was administered prophylactically, was local lymphatic vessel growth inhibited (34). Along these lines, in a corneal wounding model, lymphatic vessel regrowth kinetics accelerated with sequential challenges, indicating potential memory responses facilitating rapid recall growth in cornea (138). While it remains to be seen if similar observations can be made outside respiratory tract and cornea, these reports present the very interesting idea that long-lived lymphatic vessel aberrancies may exist, and may predict functional consequences for future inflammation, therapeutic resistance and recurrence. Clearly, if indeed refractory to normalization strategies, lymphatic vessels present an interesting challenge to clinical therapy, particularly with respect to therapeutic timing, that should be more systematically explored.

Lymphatic Vessels as an Immune Biomarker

Recent interest in the role of the immune system in tumor progression has justified a series of studies evaluating the potential for local infiltrates to provide prognostic value for risk stratification (16). Melanoma was one of the first tumors to be associated with spontaneous anti-tumor immunity leading to regression - appearance of autoimmune vitiligo (139), presence of intralesional tumor infiltrating lymphocytes (140), expression of lymphocyte chemotactic factors (141) and a type I interferon transcriptional profile (142) enrich for a subset of clinical responders in this context. This idea has been formalized into a quantitative metric reflecting immune histopathology of tumors and is a significant prognostic indicator for disease-free survival and overall survival for colorectal cancer (16,143). Evaluation of immune contexture of tumors, e.g. 'Immunoscore', includes quantification of the density of CD8⁺ and CD45RO⁺ effector and memory T cells, as a function of their location in central tumor regions as compared to invasive margins (143). Recognition that Immunoscore significantly reflects a prognostic biomarker for tumor

progression and patient outcome has yielded a broader awareness of the importance of CD8⁺ T cell infiltration as an indicator of an immunologically reactive tumor (4). What may further inform this type of immune-based stratification strategy is now an intense area of study and efforts to include various suppressive components of tumor microenvironments might provide added value to current approaches. These efforts typically focus on infiltrating immune cells of myeloid origin, however, the potential role for non-hematopoietic stromal components such as vasculature, both blood and lymph, deserves attention. In fact, it may be the close-association between tumor-associated lymphangiogenesis and infiltrating myeloid cells underlying controversy in clinical lymphatic vessel biomarker studies (39,91). This, together with evidence that the degree of both intratumoral and peritumoral lymphatic vessel density associated with the presence of tumor-associated macrophages, more advanced stage (i.e. increased tumor thickness, mitotic count and ulceration) (39) and decreased numbers of infiltrating lymphocytes (144), indicates that correlations between lymphatic vasculature and local immunity in clinical samples is significant to consider.

Current immunohistochemical mapping of immune infiltrates and other components of tumor microenvironments are necessarily reliant on biopsies of primary or metastatic disease. Solid tumors, however, are known to be heterogeneous with respect to both distribution of intrinsic oncogenic signaling and activation of microenvironmental factors (145). Consequently, biopsies may not capture the full complexity within a developing tumor. While still the most valuable approach, identification of novel tissue biomarkers must be coupled to efforts to define correlative systemic biomarker signatures. The challenge in this approach is, however, exemplified by the fact that while VEGF-C is prognostic when detected in tissue by immunohistochemistry (101), few studies have demonstrated a correlation between serum VEGF A/C/D and LN metastasis (146). VEGF A/C/D produced at high concentrations in local tissue may become diluted or unstable as they circulate through the lymphoid system back to the thoracic duct due to their short half-lives in circulation, as a consequence it is not expected that serum levels will be necessarily diagnostic of LN metastases. A potential alternative source for biomarker identification is tumor interstitial fluid itself, which, unlike plasma, has a higher concentration of these proteins produced within tumor microenvironments and therefore may more reliably reflect altered biology within the tumor itself (147). Available methods for interstitial fluid isolation have been recently reviewed and include tissue centrifugation, tissue elution, capillary ultrafiltration and microdialysis (147). Enhanced levels of known cancer biomarkers, including VEGF, can be detected in interstitial fluid when compared to plasma (147), supporting the idea that tissue interstitial fluid, the contents of which are closer to lymph than plasma, may be a more robust source of biomarkers predicting primary tumor behavior.

Conclusions

Our understanding of the roles lymphatic vessels play in regulating tumor immune microenvironments in skin and other solid tumors is still in its infancy. The interplay between the vasculature (both hematogenous and lymphogenous), local inflammation and tumor progression sets up a far more complex story than that of nutrient delivery and waste removal (Figure 4A and B). The lymphatic vasculature coordinates local inflammation and

immunity and its dysfunction may contribute to deregulated local inflammation, a hallmark of cancer as described by Hanahan and Weinberg (15). However, where lymphatic vessels really come into play in a developing cutaneous malignancy remains unclear. Whether their dysfunction early predisposes tissue to altered immunity thus leading to tumor immune escape or if their predominant role comes later during metastasis and spread remains an open question. Likely, lymphatic vessels participate in a continuous feedback loop, responding to microenvironmental change in peripheral tissue to propagate signal to lymph nodes that then alters host responses to tissue and cyclically continues. Our continued understanding of the complexities of lymphatic vessel function in the unique contexts of normal and diseased skin will provide a model to understand how regional immune dysfunction can participate in cutaneous malignancy and potential targets for prognostic and therapeutic strategies.

References

1. Pasparakis M, Haase I, Nestle FO. Mechanisms regulating skin immunity and inflammation. *Nat Rev Immunol*. 2014 Apr 11; 14(5):289–301. [PubMed: 24722477]
2. Streilein JW. Skin-associated lymphoid tissues (SALT): origins and functions. *J Invest Dermatol*. 1983 Jun; 80(Suppl):12s– 16s. [PubMed: 6602189]
3. Topalian SL, Drake CG, Pardoll DM. Immune Checkpoint Blockade: A Common Denominator Approach to Cancer Therapy. *Cancer Cell*. 2015 Apr 13; 27(4):450–61. [PubMed: 25858804]
4. Gajewski TF, Woo S-R, Zha Y, Spaapen R, Zheng Y, Corrales L, et al. Cancer immunotherapy strategies based on overcoming barriers within the tumor microenvironment. *Curr Opin Immunol*. 2013 Apr; 25(2):268–76. [PubMed: 23579075]
5. Motz GT, Coukos G. The parallel lives of angiogenesis and immunosuppression: cancer and other tales. *Nat Rev Immunol*. 2011 Oct; 11(10):702–11. [PubMed: 21941296]
6. Alitalo AK, Proulx ST, Karaman S, Aebischer D, Martino S, Jost M, et al. VEGF-C and VEGF-D blockade inhibits inflammatory skin carcinogenesis. *Cancer Res*. 2013 Jul 15; 73(14):4212–21. [PubMed: 23695550]
7. Lund AW, Duraes FV, Hirosue S, Raghavan VR, Nembrini C, Thomas SN, et al. VEGF-C promotes immune tolerance in B16 melanomas and cross-presentation of tumor antigen by lymph node lymphatics. *Cell Rep*. 2012 Mar 29; 1(3):191–9. [PubMed: 22832193]
8. Hirosue S, Vokali E, Raghavan VR, Rincon-Restrepo M, Lund AW, Corthésy-Henrioud P, et al. Steady-State Antigen Scavenging, Cross-Presentation, and CD8+ T Cell Priming: A New Role for Lymphatic Endothelial Cells. *J Immunol Baltim Md 1950*. 2014 Jun 1; 192(11):5002–11.
9. Cohen JN, Guidi CJ, Tewalt EF, Qiao H, Rouhani SJ, Ruddell A, et al. Lymph node-resident lymphatic endothelial cells mediate peripheral tolerance via Aire-independent direct antigen presentation. *J Exp Med*. 2010 Apr 12; 207(4):681–8. [PubMed: 20308365]
10. Tewalt EF, Cohen JN, Rouhani SJ, Guidi CJ, Qiao H, Fahl SP, et al. Lymphatic endothelial cells induce tolerance via PD-L1 and lack of costimulation leading to high-level PD-1 expression on CD8 T cells. *Blood*. 2012 Dec 6; 120(24):4772–82. [PubMed: 22993390]
11. Cohen JN, Tewalt EF, Rouhani SJ, Buonomo EL, Bruce AN, Xu X, et al. Tolerogenic properties of lymphatic endothelial cells are controlled by the lymph node microenvironment. *PloS One*. 2014; 9(2):e87740. [PubMed: 24503860]
12. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin*. 2010 Oct; 60(5):277–300. [PubMed: 20610543]
13. Maru GB, Gandhi K, Ramchandani A, Kumar G. The role of inflammation in skin cancer. *Adv Exp Med Biol*. 2014; 816:437–69. [PubMed: 24818733]
14. Loeb LA, Harris CC. Advances in chemical carcinogenesis: a historical review and prospective. *Cancer Res*. 2008 Sep 1; 68(17):6863–72. [PubMed: 18757397]

15. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011 Mar 4; 144(5): 646–74. [PubMed: 21376230]
16. Galon J, Mlecnik B, Bindea G, Angell HK, Berger A, Lagorce C, et al. Towards the introduction of the “Immunoscore” in the classification of malignant tumours. *J Pathol*. 2014 Jan; 232(2):199–209. [PubMed: 24122236]
17. Coussens LM, Zitvogel L, Palucka AK. Neutralizing tumor-promoting chronic inflammation: a magic bullet? *Science*. 2013 Jan 18; 339(6117):286–91. [PubMed: 23329041]
18. Proksch E, Brandner JM, Jensen J-M. The skin: an indispensable barrier. *Exp Dermatol*. 2008 Dec; 17(12):1063–72. [PubMed: 19043850]
19. Bos JD, Zonneveld I, Das PK, Krieg SR, van der Loos CM, Kapsenberg ML. The skin immune system (SIS): distribution and immunophenotype of lymphocyte subpopulations in normal human skin. *J Invest Dermatol*. 1987 May; 88(5):569–73. [PubMed: 3494791]
20. Medler TR, Coussens LM. Duality of the immune response in cancer: lessons learned from skin. *J Invest Dermatol*. 2014; 134(e1):E23–8. [PubMed: 25302470]
21. Coussens LM, Raymond WW, Bergers G, Laig-Webster M, Behrendtsen O, Werb Z, et al. Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. *Genes Dev*. 1999 Jun 1; 13(11):1382–97. [PubMed: 10364156]
22. Stockmann C, Schadendorf D, Klose R, Helfrich I. The impact of the immune system on tumor: angiogenesis and vascular remodeling. *Front Oncol*. 2014; 4:69. [PubMed: 24782982]
23. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. *Nat Med*. 2013 Nov; 19(11):1423–37. [PubMed: 24202395]
24. Swartz MA, Lund AW. Lymphatic and interstitial flow in the tumour microenvironment: linking mechanobiology with immunity. *Nat Rev Cancer*. 2012 Mar; 12(3):210–9. [PubMed: 22362216]
25. Yao L-C, Baluk P, Srinivasan RS, Oliver G, McDonald DM. Plasticity of button-like junctions in the endothelium of airway lymphatics in development and inflammation. *Am J Pathol*. 2012 Jun; 180(6):2561–75. [PubMed: 22538088]
26. Stacker SA, Williams SP, Karnezis T, Shayan R, Fox SB, Achen MG. Lymphangiogenesis and lymphatic vessel remodelling in cancer. *Nat Rev Cancer*. 2014 Mar; 14(3):159–72. [PubMed: 24561443]
27. Zheng W, Aspelund A, Alitalo K. Lymphangiogenic factors, mechanisms, and applications. *J Clin Invest*. 2014 Mar; 124(3):878–87. [PubMed: 24590272]
28. Baluk P, Fuxe J, Hashizume H, Romano T, Lashnits E, Butz S, et al. Functionally specialized junctions between endothelial cells of lymphatic vessels. *J Exp Med*. 2007 Oct 1; 204(10):2349–62. [PubMed: 17846148]
29. Carlson JA. Lymphedema and subclinical lymphostasis (microlymphedema) facilitate cutaneous infection, inflammatory dermatoses, and neoplasia: A locus minoris resistentiae. *Clin Dermatol*. 2014 Oct; 32(5):599–615. [PubMed: 25160101]
30. Kim H, Kataru RP, Koh GY. Inflammation-associated lymphangiogenesis: a double-edged sword? *J Clin Invest*. 2014 Mar 3; 124(3):936–42. [PubMed: 24590279]
31. Card CM, Yu SS, Swartz MA. Emerging roles of lymphatic endothelium in regulating adaptive immunity. *J Clin Invest*. 2014 Mar; 124(3):943–52. [PubMed: 24590280]
32. Hos D, Cursiefen C. Lymphatic vessels in the development of tissue and organ rejection. *Adv Anat Embryol Cell Biol*. 2014; 214:119–41. [PubMed: 24276891]
33. Fiedler E, Helmbold P, Marsch WC. Increased vessel density in psoriasis: involvement of lymphatic vessels in the papillary dermis. *Br J Dermatol*. 2008 Jul; 159(1):258–61. [PubMed: 18489591]
34. Yao L-C, Baluk P, Feng J, McDonald DM. Steroid-resistant lymphatic remodeling in chronically inflamed mouse airways. *Am J Pathol*. 2010 Mar; 176(3):1525–41. [PubMed: 20093490]
35. D'Alessio S, Correale C, Tacconi C, Gandelli A, Pietrogrande G, Vetrano S, et al. VEGF-C-dependent stimulation of lymphatic function ameliorates experimental inflammatory bowel disease. *J Clin Invest*. 2014 Sep 2; 124(9):3863–78. [PubMed: 25105363]
36. Kunstfeld R, Hirakawa S, Hong Y-K, Schacht V, Lange-Asschenfeldt B, Velasco P, et al. Induction of cutaneous delayed-type hypersensitivity reactions in VEGF-A transgenic mice results

- in chronic skin inflammation associated with persistent lymphatic hyperplasia. *Blood*. 2004 Aug 15; 104(4):1048–57. [PubMed: 15100155]
37. Skobe M, Hamberg LM, Hawighorst T, Schirner M, Wolf GL, Alitalo K, et al. Concurrent induction of lymphangiogenesis, angiogenesis, and macrophage recruitment by vascular endothelial growth factor-C in melanoma. *Am J Pathol*. 2001 Sep; 159(3):893–903. [PubMed: 11549582]
 38. Schoppmann SF, Birner P, Stöckl J, Kalt R, Ullrich R, Caucig C, et al. Tumor-associated macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphangiogenesis. *Am J Pathol*. 2002 Sep; 161(3):947–56. [PubMed: 12213723]
 39. Storr SJ, Safuan S, Mitra A, Elliott F, Walker C, Vasko MJ, et al. Objective assessment of blood and lymphatic vessel invasion and association with macrophage infiltration in cutaneous melanoma. *Mod Pathol Off J U S Can Acad Pathol Inc*. 2012 Apr; 25(4):493–504.
 40. Angeli V, Ginhoux F, Llodrà J, Quemeneur L, Frenette PS, Skobe M, et al. B cell-driven lymphangiogenesis in inflamed lymph nodes enhances dendritic cell mobilization. *Immunity*. 2006 Feb; 24(2):203–15. [PubMed: 16473832]
 41. Tan KW, Chong SZ, Wong FHS, Evrard M, Tan SM-L, Keeble J, et al. Neutrophils contribute to inflammatory lymphangiogenesis by increasing VEGF-A bioavailability and secreting VEGF-D. *Blood*. 2013 Nov 21; 122(22):3666–77. [PubMed: 24113869]
 42. Kataru RP, Kim H, Jang C, Choi DK, Koh BI, Kim M, et al. T lymphocytes negatively regulate lymph node lymphatic vessel formation. *Immunity*. 2011 Jan 28; 34(1):96–107. [PubMed: 21256057]
 43. Ohl L, Mohaupt M, Czeloth N, Hintzen G, Kiafard Z, Zwirner J, et al. CCR7 governs skin dendritic cell migration under inflammatory and steady-state conditions. *Immunity*. 2004 Aug; 21(2):279–88. [PubMed: 15308107]
 44. Teixeira A, Rouzaut A, Melero I. Initial afferent lymphatic vessels controlling outbound leukocyte traffic from skin to lymph nodes. *Front Immunol*. 2013; 4:433. [PubMed: 24368908]
 45. Teixeira A, Russo E, Halin C. Taking the lymphatic route: dendritic cell migration to draining lymph nodes. *Semin Immunopathol*. 2014 Mar; 36(2):261–74. [PubMed: 24402708]
 46. Lämmermann T, Bader BL, Monkley SJ, Worbs T, Wedlich-Söldner R, Hirsch K, et al. Rapid leukocyte migration by integrin-independent flowing and squeezing. *Nature*. 2008 May 1; 453(7191):51–5. [PubMed: 18451854]
 47. Miteva DO, Rutkowski JM, Dixon JB, Kilarski W, Shields JD, Swartz MA. Transmural flow modulates cell and fluid transport functions of lymphatic endothelium. *Circ Res*. 2010 Mar 19; 106(5):920–31. [PubMed: 20133901]
 48. Becker HM, Rullo J, Chen M, Ghazarian M, Bak S, Xiao H, et al. Integrin-Mediated Adhesion Inhibits Macrophage Exit from a Peripheral Inflammatory Lesion. *J Immunol*. 2013 Apr 15; 190(8):4305–14. [PubMed: 23509351]
 49. Johnson LA, Jackson DG. Control of dendritic cell trafficking in lymphatics by chemokines. *Angiogenesis*. 2014 Apr; 17(2):335–45. [PubMed: 24232855]
 50. Vigl B, Aebischer D, Nitschké M, Iolyeva M, Röthlin T, Antsiferova O, et al. Tissue inflammation modulates gene expression of lymphatic endothelial cells and dendritic cell migration in a stimulus-dependent manner. *Blood*. 2011 Jul 7; 118(1):205–15. [PubMed: 21596851]
 51. Rantakari P, Auvinen K, Jäppinen N, Kapraali M, Valtonen J, Karikoski M, et al. The endothelial protein PLVAP in lymphatics controls the entry of lymphocytes and antigens into lymph nodes. *Nat Immunol*. 2015 Apr; 16(4):386–96. [PubMed: 25665101]
 52. Sixt M, Kanazawa N, Selg M, Samson T, Roos G, Reinhardt DP, et al. The conduit system transports soluble antigens from the afferent lymph to resident dendritic cells in the T cell area of the lymph node. *Immunity*. 2005 Jan; 22(1):19–29. [PubMed: 15664156]
 53. Mantovani A, Bonecchi R, Locati M. Tuning inflammation and immunity by chemokine sequestration: decoys and more. *Nat Rev Immunol*. 2006 Dec; 6(12):907–18. [PubMed: 17124512]
 54. Jamieson T, Cook DN, Nibbs RJB, Rot A, Nixon C, McLean P, et al. The chemokine receptor D6 limits the inflammatory response in vivo. *Nat Immunol*. 2005 Apr; 6(4):403–11. [PubMed: 15750596]

55. McKimmie CS, Singh MD, Hewit K, Lopez-Franco O, Le Brocq M, Rose-John S, et al. An analysis of the function and expression of D6 on lymphatic endothelial cells. *Blood*. 2013 May 2; 121(18):3768–77. [PubMed: 23479571]
56. Lee KM, McKimmie CS, Gilchrist DS, Pallas KJ, Nibbs RJ, Garside P, et al. D6 facilitates cellular migration and fluid flow to lymph nodes by suppressing lymphatic congestion. *Blood*. 2011 Dec 1; 118(23):6220–9. [PubMed: 21979941]
57. Savino B, Caronni N, Anselmo A, Pasqualini F, Borroni EM, Basso G, et al. ERK-dependent downregulation of the atypical chemokine receptor D6 drives tumor aggressiveness in Kaposi sarcoma. *Cancer Immunol Res*. 2014 Jul; 2(7):679–89. [PubMed: 24844911]
58. Teixeira A, Garasa S, Peláez R, Azpilikueta A, Ochoa C, Marré D, et al. Lymphatic endothelium forms integrin-engaging 3D structures during DC transit across inflamed lymphatic vessels. *J Invest Dermatol*. 2013 Sep; 133(9):2276–85. [PubMed: 23528818]
59. Nitschké M, Aebischer D, Abadier M, Haener S, Lucic M, Vigl B, et al. Differential requirement for ROCK in dendritic cell migration within lymphatic capillaries in steady-state and inflammation. *Blood*. 2012 Sep 13; 120(11):2249–58. [PubMed: 22855606]
60. Dubrot J, Duraes FV, Potin L, Capotosti F, Brighouse D, Suter T, et al. Lymph node stromal cells acquire peptide-MHCII complexes from dendritic cells and induce antigen-specific CD4⁺ T cell tolerance. *J Exp Med*. 2014 Jun 2; 211(6):1153–66. [PubMed: 24842370]
61. Podgrabska S, Kamalu O, Mayer L, Shimaoka M, Snoeck H, Randolph GJ, et al. Inflamed lymphatic endothelium suppresses dendritic cell maturation and function via Mac-1/ICAM-1-dependent mechanism. *J Immunol Baltim Md 1950*. 2009 Aug 1; 183(3):1767–79.
62. Huggenberger R, Ullmann S, Proulx ST, Pytowski B, Alitalo K, Detmar M. Stimulation of lymphangiogenesis via VEGFR-3 inhibits chronic skin inflammation. *J Exp Med*. 2010 Sep 27; 207(10):2255–69. [PubMed: 20837699]
63. Kajiya K, Sawane M, Huggenberger R, Detmar M. Activation of the VEGFR-3 pathway by VEGF-C attenuates UVB-induced edema formation and skin inflammation by promoting lymphangiogenesis. *J Invest Dermatol*. 2009 May; 129(5):1292–8. [PubMed: 19005491]
64. Baluk P, Tammela T, Ator E, Lyubynska N, Achen MG, Hicklin DJ, et al. Pathogenesis of persistent lymphatic vessel hyperplasia in chronic airway inflammation. *J Clin Invest*. 2005 Feb; 115(2):247–57. [PubMed: 15668734]
65. Morton AM, Sefik E, Upadhyay R, Weissleder R, Benoist C, Mathis D. Endoscopic photoconversion reveals unexpectedly broad leukocyte trafficking to and from the gut. *Proc Natl Acad Sci U S A*. 2014 May 6; 111(18):6696–701. [PubMed: 24753589]
66. Tomura M, Honda T, Tanizaki H, Otsuka A, Egawa G, Tokura Y, et al. Activated regulatory T cells are the major T cell type emigrating from the skin during a cutaneous immune response in mice. *J Clin Invest*. 2010 Mar; 120(3):883–93. [PubMed: 20179354]
67. Jennrich S, Lee MH, Lynn RC, Dewberry K, Debes GF. Tissue exit: a novel control point in the accumulation of antigen-specific CD8 T cells in the influenza A virus-infected lung. *J Virol*. 2012 Apr; 86(7):3436–45. [PubMed: 22278253]
68. Gresser I, Guy-Grand D, Maury C, Maunoury MT. Interferon induces peripheral lymphadenopathy in mice. *J Immunol Baltim Md 1950*. 1981 Oct; 127(4):1569–75.
69. Chung HT, Samlowski WE, Kelsey DK, Daynes RA. Alterations in lymphocyte recirculation within ultraviolet light-irradiated mice: efferent blockade of lymphocyte egress from peripheral lymph nodes. *Cell Immunol*. 1986 Oct 15; 102(2):335–45. [PubMed: 3802204]
70. Bromley SK, Thomas SY, Luster AD. Chemokine receptor CCR7 guides T cell exit from peripheral tissues and entry into afferent lymphatics. *Nat Immunol*. 2005 Sep; 6(9):895–901. [PubMed: 16116469]
71. Debes GF, Arnold CN, Young AJ, Krautwald S, Lipp M, Hay JB, et al. Chemokine receptor CCR7 required for T lymphocyte exit from peripheral tissues. *Nat Immunol*. 2005 Sep; 6(9):889–94. [PubMed: 16116468]
72. Brown MN, Fintushel SR, Lee MH, Jennrich S, Geherin SA, Hay JB, et al. Chemoattractant receptors and lymphocyte egress from extralymphoid tissue: changing requirements during the course of inflammation. *J Immunol Baltim Md 1950*. 2010 Oct 15; 185(8):4873–82.

73. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. *Science*. 2015 Apr 3; 348(6230):69–74. [PubMed: 25838375]
74. Cyster JG, Schwab SR. Sphingosine-1-phosphate and lymphocyte egress from lymphoid organs. *Annu Rev Immunol*. 2012; 30:69–94. [PubMed: 22149932]
75. Pham THM, Baluk P, Xu Y, Grigorova I, Bankovich AJ, Pappu R, et al. Lymphatic endothelial cell sphingosine kinase activity is required for lymphocyte egress and lymphatic patterning. *J Exp Med*. 2010 Jan 18; 207(1):17–27. [PubMed: 20026661]
76. Ledgerwood LG, Lal G, Zhang N, Garin A, Esses SJ, Ginhoux F, et al. The sphingosine 1-phosphate receptor 1 causes tissue retention by inhibiting the entry of peripheral tissue T lymphocytes into afferent lymphatics. *Nat Immunol*. 2008 Jan; 9(1):42–53. [PubMed: 18037890]
77. Lukacs-Kornek V, Malhotra D, Fletcher AL, Acton SE, Elpek KG, Tayalia P, et al. Regulated release of nitric oxide by nonhematopoietic stroma controls expansion of the activated T cell pool in lymph nodes. *Nat Immunol*. 2011 Nov; 12(11):1096–104. [PubMed: 21926986]
78. Maio M. Melanoma as a model tumour for immuno-oncology. *Ann Oncol Off J Eur Soc Med Oncol ESMO*. 2012 Sep; 23(Suppl 8):viii10–4.
79. Madore J, Vilain R, Menzies AM, Kakavand H, Wilmott JS, Hyman J, et al. PD-L1 expression in melanoma shows marked heterogeneity within and between patients: implications for anti-PD-1/PD-L1 clinical trials. *Pigment Cell Melanoma Res*. 2014 Dec 5.
80. Tamburini BA, Burchill MA, Kedl RM. Antigen capture and archiving by lymphatic endothelial cells following vaccination or viral infection. *Nat Commun*. 2014; 5:3989. [PubMed: 24905362]
81. Ruocco V, Ruocco E, Piccolo V, Brunetti G, Guerrera LP, Wolf R. The immunocompromised district in dermatology: A unifying pathogenic view of the regional immune dysregulation. *Clin Dermatol*. 2014 Oct; 32(5):569–76. [PubMed: 25160098]
82. Nishigori C, Yarosh DB, Donawho C, Kripke ML. The immune system in ultraviolet carcinogenesis. *J Invest Dermatol Symp Proc Soc Invest Dermatol Inc Eur Soc Dermatol Res*. 1996 Apr; 1(2):143–6.
83. Kajiya K, Kunstfeld R, Detmar M, Chung JH. Reduction of lymphatic vessels in photodamaged human skin. *J Dermatol Sci*. 2007 Sep; 47(3):241–3. [PubMed: 17572071]
84. Back SJ, Kim YJ, Choi DK, Lee Y, Seo YJ, Park JK, et al. Cutaneous lymphangiectasia associated with photoageing and topical corticosteroid application. *Clin Exp Dermatol*. 2009 Apr; 34(3):352–4. [PubMed: 19175787]
85. Matsumoto-Okazaki Y, Furuse M, Kajiya K. Claudin-5 haploinsufficiency exacerbates UVB-induced oedema formation by inducing lymphatic vessel leakage. *Exp Dermatol*. 2012 Jul; 21(7):557–9. [PubMed: 22716257]
86. Kajiya K, Hirakawa S, Detmar M. Vascular endothelial growth factor-A mediates ultraviolet B-induced impairment of lymphatic vessel function. *Am J Pathol*. 2006 Oct; 169(4):1496–503. [PubMed: 17003502]
87. Kajiya K, Kidoya H, Sawane M, Matsumoto-Okazaki Y, Yamanishi H, Furuse M, et al. Promotion of lymphatic integrity by angiopoietin-1/Tie2 signaling during inflammation. *Am J Pathol*. 2012 Mar; 180(3):1273–82. [PubMed: 22200616]
88. Ruocco V, Brunetti G, Puca RV, Ruocco E. The immunocompromised district: a unifying concept for lymphoedematous, herpes-infected and otherwise damaged sites. *J Eur Acad Dermatol Venereol JEADV*. 2009 Dec; 23(12):1364–73. [PubMed: 19548975]
89. Bryant-Hudson KM, Chucair-Elliott AJ, Conrady CD, Cohen A, Zheng M, Carr DJJ. HSV-1 targets lymphatic vessels in the eye and draining lymph node of mice leading to edema in the absence of a functional type I interferon response. *Am J Pathol*. 2013 Oct; 183(4):1233–42. [PubMed: 23911821]
90. Conrady CD, Zheng M, Stone DU, Carr DJJ. CD8+ T cells suppress viral replication in the cornea but contribute to VEGF-C-induced lymphatic vessel genesis. *J Immunol Baltim Md 1950*. 2012 Jul 1; 189(1):425–32.
91. Moussai D, Mitsui H, Pettersen JS, Pierson KC, Shah KR, Suárez-Fariñas M, et al. The human cutaneous squamous cell carcinoma microenvironment is characterized by increased lymphatic density and enhanced expression of macrophage-derived VEGF-C. *J Invest Dermatol*. 2011 Jan; 131(1):229–36. [PubMed: 20827282]

92. Eichten A, Hyun WC, Coussens LM. Distinctive features of angiogenesis and lymphangiogenesis determine their functionality during de novo tumor development. *Cancer Res.* 2007 Jun 1; 67(11): 5211–20. [PubMed: 17545601]
93. Honkonen KM, Visuri MT, Tervala TV, Halonen PJ, Koivisto M, Lähteenvuo MT, et al. Lymph node transfer and perinodal lymphatic growth factor treatment for lymphedema. *Ann Surg.* 2013 May; 257(5):961–7. [PubMed: 23013803]
94. Pasquali S, van der Ploeg APT, Mocellin S, Stretch JR, Thompson JF, Scolyer RA. Lymphatic biomarkers in primary melanomas as predictors of regional lymph node metastasis and patient outcomes. *Pigment Cell Melanoma Res.* 2013 May; 26(3):326–37. [PubMed: 23298266]
95. Achen MG, Jeltsch M, Kuk E, Mäkinen T, Vitali A, Wilks AF, et al. Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). *Proc Natl Acad Sci U S A.* 1998 Jan 20; 95(2):548–53. [PubMed: 9435229]
96. Joukov V, Pajusola K, Kaipainen A, Chilov D, Lahtinen I, Kuk E, et al. A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases. *EMBO J.* 1996 Apr 1; 15(7):1751. [PubMed: 8612600]
97. Skobe M, Hawighorst T, Jackson DG, Prevo R, Janes L, Velasco P, et al. Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. *Nat Med.* 2001 Feb; 7(2):192–8. [PubMed: 11175850]
98. He Y, Rajantie I, Pajusola K, Jeltsch M, Holopainen T, Ylä-Herttuala S, et al. Vascular endothelial cell growth factor receptor 3-mediated activation of lymphatic endothelium is crucial for tumor cell entry and spread via lymphatic vessels. *Cancer Res.* 2005 Jun 1; 65(11):4739–46. [PubMed: 15930292]
99. Mandriota SJ, Jussila L, Jeltsch M, Compagni A, Baetens D, Prevo R, et al. Vascular endothelial growth factor-C-mediated lymphangiogenesis promotes tumour metastasis. *EMBO J.* 2001 Feb 15; 20(4):672–82. [PubMed: 11179212]
100. Karpanen T, Egeblad M, Karkkainen MJ, Kubo H, Ylä-Herttuala S, Jäättelä M, et al. Vascular endothelial growth factor C promotes tumor lymphangiogenesis and intralymphatic tumor growth. *Cancer Res.* 2001 Mar 1; 61(5):1786–90. [PubMed: 11280723]
101. Stacker SA, Williams RA, Achen MG. Lymphangiogenic growth factors as markers of tumor metastasis. *APMIS Acta Pathol Microbiol Immunol Scand.* 2004 Aug; 112(7–8):539–49.
102. Stacker SA, Caesar C, Baldwin ME, Thornton GE, Williams RA, Prevo R, et al. VEGF-D promotes the metastatic spread of tumor cells via the lymphatics. *Nat Med.* 2001 Feb; 7(2):186–91. [PubMed: 11175849]
103. Hirakawa S, Kodama S, Kunstfeld R, Kajiya K, Brown LF, Detmar M. VEGF-A induces tumor and sentinel lymph node lymphangiogenesis and promotes lymphatic metastasis. *J Exp Med.* 2005 Apr 4; 201(7):1089–99. [PubMed: 15809353]
104. Xu Y, Yuan L, Mak J, Pardanaud L, Caunt M, Kasman I, et al. Neuropilin-2 mediates VEGF-C-induced lymphatic sprouting together with VEGFR3. *J Cell Biol.* 2010 Jan 11; 188(1):115–30. [PubMed: 20065093]
105. He Y, Kozaki K-I, Karpanen T, Koshikawa K, Ylä-Herttuala S, Takahashi T, et al. Suppression of tumor lymphangiogenesis and lymph node metastasis by blocking vascular endothelial growth factor receptor 3 signaling. *J Natl Cancer Inst.* 2002 Jun 5; 94(11):819–25. [PubMed: 12048269]
106. Dadras SS, Paul T, Bertoncini J, Brown LF, Muzikansky A, Jackson DG, et al. Tumor lymphangiogenesis: a novel prognostic indicator for cutaneous melanoma metastasis and survival. *Am J Pathol.* 2003 Jun; 162(6):1951–60. [PubMed: 12759251]
107. Shayan R, Karnezis T, Murali R, Wilmott JS, Ashton MW, Taylor GI, et al. Lymphatic vessel density in primary melanomas predicts sentinel lymph node status and risk of metastasis. *Histopathology.* 2012 Oct; 61(4):702–10. [PubMed: 22734806]
108. Wobser M, Siedel C, Schrama D, Bröcker E-B, Becker JC, Vetter-Kauczok CS. Expression pattern of the lymphatic and vascular markers VEGFR-3 and CD31 does not predict regional lymph node metastasis in cutaneous melanoma. *Arch Dermatol Res.* 2006 Feb; 297(8):352–7. [PubMed: 16395613]

109. Sahni D, Robson A, Orchard G, Szydlo R, Evans AV, Russell-Jones R. The use of LYVE-1 antibody for detecting lymphatic involvement in patients with malignant melanoma of known sentinel node status. *J Clin Pathol*. 2005 Jul; 58(7):715–21. [PubMed: 15976338]
110. Werchau S, Toberer F, Enk A, Dammann R, Helmbold P. Merkel cell carcinoma induces lymphatic microvessel formation. *J Am Acad Dermatol*. 2012 Aug; 67(2):215–25. [PubMed: 22050913]
111. Karpova MB, Fujii K, Jenni D, Dummer R, Urosevic-Maiwald M. Evaluation of lymphangiogenic markers in Sézary syndrome. *Leuk Lymphoma*. 2011 Mar; 52(3):491–501. [PubMed: 20846094]
112. Qian C-N, Berghuis B, Tsarfaty G, Bruch M, Kort EJ, Ditlev J, et al. Preparing the “soil”: the primary tumor induces vasculature reorganization in the sentinel lymph node before the arrival of metastatic cancer cells. *Cancer Res*. 2006 Nov 1; 66(21):10365–76. [PubMed: 17062557]
113. Gogineni A, Caunt M, Crow A, Lee CV, Fuh G, van Bruggen N, et al. Inhibition of VEGF-C modulates distal lymphatic remodeling and secondary metastasis. *PloS One*. 2013; 8(7):e68755. [PubMed: 23874750]
114. Hoshida T, Isaka N, Hagendoorn J, di Tomaso E, Chen Y-L, Pytowski B, et al. Imaging steps of lymphatic metastasis reveals that vascular endothelial growth factor-C increases metastasis by increasing delivery of cancer cells to lymph nodes: therapeutic implications. *Cancer Res*. 2006 Aug 15; 66(16):8065–75. [PubMed: 16912183]
115. Harrell MI, Iritani BM, Ruddell A. Tumor-induced sentinel lymph node lymphangiogenesis and increased lymph flow precede melanoma metastasis. *Am J Pathol*. 2007 Feb; 170(2):774–86. [PubMed: 17255343]
116. Proulx ST, Luciani P, Derzsi S, Rinderknecht M, Mumprecht V, Leroux J-C, et al. Quantitative imaging of lymphatic function with liposomal indocyanine green. *Cancer Res*. 2010 Sep 15; 70(18):7053–62. [PubMed: 20823159]
117. Hofmann M, Pflanzner R, Zoller NN, Bernd A, Kaufmann R, Thaci D, et al. Vascular endothelial growth factor C-induced lymphangiogenesis decreases tumor interstitial fluid pressure and tumor. *Transl Oncol*. 2013 Aug; 6(4):398–404. [PubMed: 23908682]
118. Padera TP, Kadambi A, di Tomaso E, Carreira CM, Brown EB, Boucher Y, et al. Lymphatic metastasis in the absence of functional intratumor lymphatics. *Science*. 2002 Jun 7; 296(5574):1883–6. [PubMed: 11976409]
119. Wong SY, Haack H, Crowley D, Barry M, Bronson RT, Hynes RO. Tumor-secreted vascular endothelial growth factor-C is necessary for prostate cancer lymphangiogenesis, but lymphangiogenesis is unnecessary for lymph node metastasis. *Cancer Res*. 2005 Nov 1; 65(21):9789–98. [PubMed: 16267000]
120. Ran S, Volk L, Hall K, Flister MJ. Lymphangiogenesis and lymphatic metastasis in breast cancer. *Pathophysiology*. 2010 Sep; 17(4):229–51. [PubMed: 20036110]
121. Siironen P, Ristimäki A, Narko K, Nordling S, Louhimo J, Andersson S, et al. VEGF-C and COX-2 expression in papillary thyroid cancer. *Endocr Relat Cancer*. 2006 Jun; 13(2):465–73. [PubMed: 16728574]
122. Soumaoro LT, Uetake H, Takagi Y, Iida S, Higuchi T, Yasuno M, et al. Coexpression of VEGF-C and Cox-2 in human colorectal cancer and its association with lymph node metastasis. *Dis Colon Rectum*. 2006 Mar; 49(3):392–8. [PubMed: 16474989]
123. Timoshenko AV, Chakraborty C, Wagner GF, Lala PK. COX-2-mediated stimulation of the lymphangiogenic factor VEGF-C in human breast cancer. *Br J Cancer*. 2006 Apr 24; 94(8):1154–63. [PubMed: 16570043]
124. Zhang X-H, Huang D-P, Guo G-L, Chen G-R, Zhang H-X, Wan L, et al. Coexpression of VEGF-C and COX-2 and its association with lymphangiogenesis in human breast cancer. *BMC Cancer*. 2008; 8:4. [PubMed: 18190720]
125. Hosono K, Suzuki T, Tamaki H, Sakagami H, Hayashi I, Narumiya S, et al. Roles of prostaglandin E2-EP3/EP4 receptor signaling in the enhancement of lymphangiogenesis during fibroblast growth factor-2-induced granulation formation. *Arterioscler Thromb Vasc Biol*. 2011 May; 31(5):1049–58. [PubMed: 21311040]

126. Su J-L, Shih J-Y, Yen M-L, Jeng Y-M, Chang C-C, Hsieh C-Y, et al. Cyclooxygenase-2 induces EP1- and HER-2/Neu-dependent vascular endothelial growth factor-C up-regulation: a novel mechanism of lymphangiogenesis in lung adenocarcinoma. *Cancer Res.* 2004 Jan 15; 64(2):554–64. [PubMed: 14744769]
127. Lyons TR, Borges VF, Betts CB, Guo Q, Kapoor P, Martinson HA, et al. Cyclooxygenase-2-dependent lymphangiogenesis promotes nodal metastasis of postpartum breast cancer. *J Clin Invest.* 2014 Sep; 124(9):3901–12. [PubMed: 25133426]
128. Karnezis T, Shayan R, Caesar C, Roufail S, Harris NC, Ardipradja K, et al. VEGF-D promotes tumor metastasis by regulating prostaglandins produced by the collecting lymphatic endothelium. *Cancer Cell.* 2012 Feb 14; 21(2):181–95. [PubMed: 22340592]
129. Rodriguez-Burford C, Tu JH, Mercurio M, Carey D, Han R, Gordon G, et al. Selective cyclooxygenase-2 inhibition produces heterogeneous erythema response to ultraviolet irradiation. *J Invest Dermatol.* 2005 Dec; 125(6):1317–20. [PubMed: 16354205]
130. Elmetts CA, Ledet JJ, Athar M. Cyclooxygenases: mediators of UV-induced skin cancer and potential targets for prevention. *J Invest Dermatol.* 2014 Oct; 134(10):2497–502. [PubMed: 24804836]
131. Tang JY, Aszterbaum M, Athar M, Barsanti F, Cappola C, Estevez N, et al. Basal cell carcinoma chemoprevention with nonsteroidal anti-inflammatory drugs in genetically predisposed PTCH1+/- humans and mice. *Cancer Prev Res Phila Pa.* 2010 Jan; 3(1):25–34.
132. Elmetts CA, Viner JL, Pentland AP, Cantrell W, Lin H-Y, Bailey H, et al. Chemoprevention of nonmelanoma skin cancer with celecoxib: a randomized, double-blind, placebo-controlled trial. *J Natl Cancer Inst.* 2010 Dec 15; 102(24):1835–44. [PubMed: 21115882]
133. Welte J, Loges S, Dimmeler S, Carmeliet P. Recent molecular discoveries in angiogenesis and antiangiogenic therapies in cancer. *J Clin Invest.* 2013 Aug; 123(8):3190–200. [PubMed: 23908119]
134. Tammela T, Zarkada G, Nurmi H, Jakobsson L, Heinolainen K, Tvorogov D, et al. VEGFR-3 controls tip to stalk conversion at vessel fusion sites by reinforcing Notch signalling. *Nat Cell Biol.* 2011 Oct; 13(10):1202–13. [PubMed: 21909098]
135. Rivera LB, Bergers G. Intertwined regulation of angiogenesis and immunity by myeloid cells. *Trends Immunol.* 2015 Apr; 36(4):240–9. [PubMed: 25770923]
136. Xin X, Majumder M, Girish GV, Mohindra V, Maruyama T, Lala PK. Targeting COX-2 and EP4 to control tumor growth, angiogenesis, lymphangiogenesis and metastasis to the lungs and lymph nodes in a breast cancer model. *Lab Invest J Tech Methods Pathol.* 2012 Aug; 92(8):1115–28.
137. Karnezis T, Shayan R, Fox S, Achen MG, Stacker SA. The connection between lymphangiogenic signalling and prostaglandin biology: a missing link in the metastatic pathway. *Oncotarget.* 2012 Aug; 3(8):893–906. [PubMed: 23097685]
138. Kelley PM, Connor AL, Tempero RM. Lymphatic vessel memory stimulated by recurrent inflammation. *Am J Pathol.* 2013 Jun; 182(6):2418–28. [PubMed: 23578386]
139. Albert DM, Todes-Taylor N, Wagoner M, Nordlund JJ, Lerner AB. Vitiligo or halo nevi occurring in two patients with choroidal melanoma. *Arch Dermatol.* 1982 Jan; 118(1):34–6. [PubMed: 7059198]
140. Cipponi A, Wieers G, van Baren N, Coulie PG. Tumor-infiltrating lymphocytes: apparently good for melanoma patients But why? *Cancer Immunol Immunother CII.* 2011 Aug; 60(8):1153–60. [PubMed: 21553145]
141. Harlin H, Meng Y, Peterson AC, Zha Y, Tretiakova M, Slingluff C, et al. Chemokine expression in melanoma metastases associated with CD8+ T-cell recruitment. *Cancer Res.* 2009 Apr 1; 69(7):3077–85. [PubMed: 19293190]
142. Fuertes MB, Kacha AK, Kline J, Woo S-R, Kranz DM, Murphy KM, et al. Host type I IFN signals are required for antitumor CD8+ T cell responses through CD8{alpha}+ dendritic cells. *J Exp Med.* 2011 Sep 26; 208(10):2005–16. [PubMed: 21930765]
143. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science.* 2006 Sep 29; 313(5795):1960–4. [PubMed: 17008531]

144. Azimi F, Scolyer RA, Rumcheva P, Moncrieff M, Murali R, McCarthy SW, et al. Tumor-infiltrating lymphocyte grade is an independent predictor of sentinel lymph node status and survival in patients with cutaneous melanoma. *J Clin Oncol Off J Am Soc Clin Oncol*. 2012 Jul 20; 30(21):2678–83.
145. Jamal-Hanjani M, Thanopoulou E, Peggs KS, Quezada SA, Swanton C. Tumour heterogeneity and immune-modulation. *Curr Opin Pharmacol*. 2013 Aug; 13(4):497–503. [PubMed: 23664091]
146. Zhang Y, Meng X, Zeng H, Guan Y, Zhang Q, Guo S, et al. Serum vascular endothelial growth factor-C levels: A possible diagnostic marker for lymph node metastasis in patients with primary non-small cell lung cancer. *Oncol Lett*. 2013 Aug; 6(2):545–9. [PubMed: 24137365]
147. Wagner M, Wiig H. Tumor Interstitial fluid formation, characterization and clinical implications. *Front Oncol*. 2015; 5(115)

Statement of Significance

The tumor microenvironment and tumor-associated inflammation are now appreciated for not only their role in cancer progression but also response to therapy. The lymphatic vasculature is a less-appreciated component of this microenvironment that coordinates local inflammation and immunity and thereby critically shapes local responses. A mechanistic understanding for the complexities of lymphatic vessel function in the unique context of skin provides a model to understand how regional immune dysfunction drives cutaneous malignancies, and as such lymphatic vessels represent a biomarker of cutaneous immunity that may provide insight into cancer prognosis and effective therapy.

Figure 1. Structure and function of initial and collecting lymphatic vessels

(A) The lymphatic vessels of skin are composed of two plexuses one superficial extending into the dermal papillae near the subpapillary arterial network, which drains vertically into the deep lymphatic plexus below the second arterial network. (B) Initial lymphatic capillaries are blind-ended vessels with discontinuous basement membrane and no associated smooth muscle cells (SMC). At resting state, the lymphatic endothelial cells that comprise the initial capillaries are characterized by unique overlapping, button-like junctions that allow for passive flow and leukocyte trafficking through interendothelial gaps in an integrin-independent manner. Local inflammation, results in vascular leakiness driving increased interstitial fluid pressures (IFP) and enhanced flows. At least in mouse respiratory tract, inflammation is associated with a remodeling of the interendothelial junctions of initial capillaries into tight, zipper-like junctions. Lymphatic capillaries are anchored directly to the

extracellular matrix (ECM) through anchoring filaments, such that under high levels of IFP, stretching of ECM results in distension of initial capillaries and enhanced fluid flows and cellular trafficking both by intercellular and transcellular mechanisms. (C) Collecting vessels are larger vessels that have both a continuous basement membrane and SMC coverage. Collecting vessels are notably defined by the presence of a system of valves, which separates the vessel into functional units or lymphangions. SMCs mediate contraction of individual lymphangions that drives the opening of downstream valves while closely valves immediate upstream. This system of local contraction and relaxation drives unidirectional fluid flows from peripheral tissues to draining lymph nodes.

Figure 2. Lymphatic vessels, inflammation and immunity

(A) Homeostatic lymphatic capillaries support immune surveillance through steady-state homing of resident immune cells, including dendritic cells (DC) and some subsets of memory T cells. (B) Local inflammation and damage activate a series of danger signaling as well as increased interstitial fluid pressures (IFP) that activate initial lymphatic capillaries resulting in remodeling (either proliferative or non-proliferative), upregulation of adhesion molecules and enhanced expression of the homing chemokine, CCL21. Altered adhesions and CCL21 coordinate to facilitate entry of activated CCR7⁺ DCs into afferent lymphatic

vessels and migration towards draining lymph nodes where they interact with and activate naïve T cells. The decoy receptor, D6, ensures proper presentation of homeostatic chemokines by LECs by scavenging inflammatory chemokines to specifically facilitate mature over immature DC migration. Changes in lymphatic flows that result from altered signaling in both initial capillaries and collecting vessels may influence accumulation of inflammatory cytokines that help to perpetuate local inflammation leading to infiltration and accumulation of leukocytes in tissue, which further drive lymphatic remodeling. **(C)** Though important for immune induction, evidence also indicates that lymphatic capillaries importantly regulate resolution of local inflammation and immunity through leukocyte egress and chemokine sequestration. Both macrophages and some T cells exit peripheral tissue through draining lymphatic capillaries using CCL21 and sphingosine kinase (SPHK) conversion of sphingosine into Sphingosine-1-P (S1P) as signals for their exit, all produced by initial lymphatic vessels. Cellular exit is required for resolution of disease. **(D)** Novel immunomodulatory roles of lymphatic endothelial cells (LEC) have been described, largely in the context of lymphoid organs. LECs inhibit both antigen-dependent and independent T cell activation through production of nitric oxide (NO) and non-specific inhibition of DC/T cell interactions. Inflamed LECs inhibit maturation of DCs through ICAM-1 and receive peptide-loaded MHCII complexes from mature DCs. Additionally, LECs promiscuously present endogenous and scavenge exogenous antigen for cross-presentation on MHCI molecules and direct deletion of antigen-specific CD8 T cells.

Figure 3. Deregulation of lymphatic vessel function, inflammation and skin carcinogenesis by environmental factors

Environmental factors that predispose to skin cancer (ultraviolet radiation, infection and surgery or physical trauma) simultaneously impact lymphatic vessel dysfunction. Lymphatic remodeling as a result of ultraviolet exposure, infection or surgery, may result in altered fluid flows and local inflammation that generate a local microenvironment more permissive to the oncogenic effects of the agents.

Figure 4. Proposed model for feedback between lymphatic vessels, inflammation and skin carcinogenesis: implications for immunotherapy

(A) Tumor-promoting inflammation induces the initiation and progression of skin cancer as well as remodeling of local lymphatic vessels, which in turn may be further tumor-promoting by facilitating the resolution response characterized by immune suppressive leukocyte infiltrates and local tolerance. Lymphatic remodeling results in enhanced fluid flows to draining lymph nodes facilitating metastatic progression of developing skin cancers. Furthermore, in addition to the pro-tumor, suppressive inflammation, lymphatic vessels may directly inhibit anti-tumor immunity preventing local control of the growing tumor, though whether this would occur in tumor microenvironments or their draining lymph nodes remains unknown. (B) Immunotherapy endeavors to switch the balance in this network toward anti-tumor immunity through methods of both direct and indirect activation of adaptive immune responses against tumors. Enhanced anti-tumor immunity will control primary growth but may also influence local remodeling of lymphatic vessels through an interferon- γ -dependent mechanism. Mechanisms of resistance to this approach have already been described where infiltrating leukocytes impair local T cell infiltration and function. Given the novel immunomodulatory roles of lymphatic vessels it remains to be seen as to

whether their status may be additionally predictive of response or alternatively a targetable mechanism of resistance.

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