Peer Review File

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Reviewer A

Comment 1：Several key statements require evidence-based references, including:
L49 "Though most solid tumors disseminate through lymphatic vessels";
L196 (214) "As increased lymphangiogenesis is correlated with a higher rate of tumor metastasis";
L315 (340) "The lymph vessels can serve as conduits for promoting tumor cell metastasis"
Reply: Thanks very much for pointing this. We have added following references supporting the mentioned statement, which were shown as follows, and the line number is quoted as it is shown in the revised manuscript.
Line 49 "Though most solid tumors disseminate through lymphatic vessels before hematogenous metastasis(Mikada, Sukhbaatar et al. 2017)";
L196 (214) "As increased lymphangiogenesis is correlated with a higher rate of tumor metastasis (Stacker, Achen et al. 2002, Mumprecht and Detmar 2009) ";
L 315(340) "The lymph vessels can serve as conduits for promoting tumor cell metastasis (Sleeman and Thiele 2009, Kerjaschki 2014)"

Comment 2. Rather than just citing references, specific molecules or techniques should be explicitly spelled out in the text, so the readers can have an idea what the authors refer to, For instance:
L318(344) "NSCLC" and "melanoma" should replace "various types of cancer" (according to ref 2 &77);
L367(392) "modification of the drug administrating route, such as [the novel lymphatic targeting delivering system], has been developed, but its efficacy in clinical practice needs to be determined in future studies (94-97)".
ref 94: [Liposomes] to target the lymphatics by subcutaneous administration. 2001
ref 95: [Epirubicin-loaded polymeric micelles] effectively treat axillary lymph nodes metastasis of breast cancer through selective accumulation and pH-triggered drug release. 2018
ref 96: [MPEG-DSPE polymeric micelle] for translymphatic chemotherapy of lymph node metastasis. 2015
ref 97: Engineering [polymer hydrogel nanoparticles] for lymph node-targeted delivery. 2016
Reply：Thanks very much for pointing out this. We have rewritten the mentioned sentences as follows:
L318 (344)" The association between lymphangiogenesis and tumor metastasis is proven for NSCLC and melanoma, in which the incidence of intratumoural and peritumoral lymphatics is correlated with lymph node invasion and poor overall survival (Dadras, Paul et al. 2003, Renyi-Vamos, Tovari et al. 2005).”
L367 (392) " such as modification of the drug administrating route has been developed. The novel lymphatic targeting delivering systems include liposomes to target the lymphatics by

subcutaneous administration, epirubicin-loaded polymeric micelles to effectively treat axillary lymph nodes metastasis of breast cancer through selective accumulation and pH-triggered drug release, MPEG-DSPE polymeric micelle for translymphatic chemotherapy of lymph node metastasis and engineering polymer hydrogel nanoparticles for lymph node-targeted delivery. But their efficacy in clinical practice needs to be determined in future studies (Oussoren and Storm 2001, Li, Dong et al. 2015, De Koker, Cui et al. 2016, Chida, Miura et al. 2018). ".

Comment 3： L369-375(399-405), please double-check the content with ref 98, that ICI treatment reinvigorates exhausted T cells, not activates naive T cells. The current statement is mis-leading.

Reply: Thanks very much for pointing out this. In this statement, we do not mean that the naive T cells are activated by ICI treatment, and we have rewritten the mentioned sentences as follows:

Line 403: These naive T cells will then be locally activated, leading to the upregulation of cytotoxic T cells in the tumor microenvironment (Fankhauser, Broggi et al. 2017).

Comment 4： L131-L133 (136-138), duplicated sentences are found.

Reply: Thanks very much for pointing out this. We have rewritten the mentioned sentences as follows:

Line 136-138: While the classical view of lymphatic metastasis is a passive transport of tumor cells under lymphatic fluid pressure, it is now confirmed that lymphatic metastasis is more likely the result of a mutual interaction between tumor cells and tumor microenvironment, such as the LECs.

Reviewer B

In the present review article, He et al, summarize the literature regarding the role of lymphatic endothelial cells and lymphangiogenesis in cancers, with special emphasis in lung cancer. The authors include information about lymphatic metastasis, lymphatic markers, growth factors and other mediators, such as ECM and inflammation and the role of lymphangiogenesis in cancer metastasis and anti-cancer treatment. Significant part of the review article is devoted in the interaction between the lymphatic endothelium and the tumor microenvironment, including the endothelial-immune cell interplay. Overall, the authors provide an in-depth and interesting insight on the role of the lymphatic endothelial cells in tumor microenvironment, highlighting interesting areas requiring further research. However, some stipulations remain, that could improve the quality of the manuscript:

Comment 1：• Line 105: A schematic describing the ceiling and parenchyma LECs would be helpful. Overall, that part is a bit hard to follow. Some re-structuring could be beneficial.

Reply： Thanks very much for pointing out this. We have rewritten this part as follows:

Line 105: In recent years, the lymphatic structure in TDLNs has also gained more attention. Within the lymph node, the afferent lymphatics transform into subcapsular sinus systems. The subcapsular sinus usually overlays the whole LN cortex, and is lined by LEC layers on both

sides. Those facing the capsule are called ceiling LECs, and those overlaying the parenchyma are called floor LECs (Sainte-Marie 2010). The subcapsular sinus is hence the first place in TDLNs met by tumor cells and leukocytes. Apart from migrating tumor cells and leukocytes, subcapsular sinus is also dwelled by resident immune cells, such as resident macrophages and dendritic cells (Gerner, Torabi-Parizi et al. 2015, Louie and Liao 2019), which are usually interspersed with floor LECs. The migratory, as well as resident leukocytes, need to transverse floor LECs to initiate immune response of effector cells in the subcapsular niche. Recent evidence also showed that LEC-produced growth factors and chemokines are essential for the development of subcapsular macrophages (Mondor, Baratin et al. 2019, Moran, Grootveld et al. 2019). On the hilum side of LNs, the subcapsular sinus is replaced by the medullary sinus, which will finally converge into the efferent lymphatic vessels for drainage away from LN. In this sense, efferent leukocytes need to travel across the LECs at the medullary sinus boundary. Tumor cells also need to infiltrate LN parenchyma across floor LECs, however, their choice of exiting routes from LN are still unclear (Brown, Assen et al. 2018). Besides subcapsular and medullary sinuses, two other types of sinuses exist, namely the transverse and cortical sinuses, which will merge into the medullary sinuses. The transverse sinuses are invaginations of the subcapsular sinus(Sainte-Marie, Peng et al. 1982), and cortical sinuses are blind-ended sacs in the paracortical zone (Grigorova, Panteleev et al. 2010). However, their exact roles in tumor metastasis are still not fully defined.

Comment 2： • some parts in the introduction require references.

Reply: Thanks very much for pointing out this. We have added necessary references in the introduction part to make it clearer, which is shown as follows.

Line 49-65:Though most solid tumors disseminate through lymphatic vessels before hematogenous metastasis (Mikada, Sukhbaatar et al. 2017), the lymphatic system is relatively overlooked compared to the tumor microenvironment blood vessels. Traditionally, the lymphatic vasculature has been considered a draining route for interstitial fluid and migrating leukocytes and tumor cells. The lymphatic endothelial cell (LEC) is the primary cell that builds lymphatic vasculature, forming the wall of lymphatic vessels which transport the fluid and migrating cells inside (Stacker, Williams et al. 2014). In the tumor lymphatic metastasis, invaded malignant cells need to squeeze between LEC junctions and move along LECs towards lymph nodes and distant organs. Thus, LECs are an essential part of the tumor micro-environment, facilitating cancer invasion and may be harnessed by tumor cells (Jiang 2019). Indeed, LEC infiltration and lymphatic vessel formation are observed frequently in a highly active tumor, and this process is called tumor-associated lymphangiogenesis (Dieterich and Detmar 2016). Recent advances also revealed that, besides this bystander role, LECs could function in multiple other ways during tumor progression, tumor-stimulating or inhibiting, and interaction with tumor cells, immune cells, or tumor stroma (Yeo and Angeli 2017, Munir, Mazzaglia et al. 2020, Lee, Jang et al. 2021). These interactions can also occur in both peripheral lymphatics and the tumor-draining lymph nodes (TDLNs) (Jalkanen and Salmi 2020), implying the existence of distinct populations of LECs and the context-dependent interaction. In this sense, LECs' complex roles in the tumor micro-environment and recent

advances in this field call for an in-depth inspection.

Comment 3:• Line 131(136): the following sentence is not clear: “it is now confirmed that mutual interaction between tumor cells and tumor microenvironment is more likely that a mutual interaction between tumor cells and tumor microenvironment”, please rephrase.
Reply: Thanks very much for pointing out this. We have rewritten this part as follows:
Line 136:While the classical view of lymphatic metastasis is a passive transport of tumor cells under lymphatic fluid pressure, it is now confirmed that lymphatic metastasis is more likely the result of a mutual interaction between tumor cells and tumor microenvironment, such as the LECs.

Comment 4:• Lines 619-620(667-668): a reference is missing regarding the chloroquine study.
Reply: Thanks very much for pointing out this. We have added reference for this part, which is shown as follows:
619-620(667-668): Indeed, by inhibiting the LEC autophagy and permeability caused by paclitaxel, chloroquine reduces tumor cell LN metastasis and increases treatment efficacy (Zamora, Alves et al. 2019).

Comment 5:• Some of the typos identified throughout the text are following: Line 72: “summary”; line 90(88): “lymph nodes (LNs)”; line 102: “subpopulations.”; line 104(106): “LEC layers”; line 161(171): “determining”; line 190 (208): “for in vitro”; line 201(219): “. VEGF-C,”; line 236 (260): “fibroblasts”; line 240(264): “leads”; line 257(280): “pathways”; line 265(289): “inflammation and carcinogenesis”; line 285(311): “integrins”; line 286(312): “are necessary”; linen 301(327): “regulated”; line 310(336): “afterwards”; line 318(344): “proven”; line 355(380): “can be found”; line 385(414): “indeed proven”; line 417(445): “metastasis.”; line 501(533): “Research showed”; line 584(625): “in the clinic”; line 585(626): “have gained”; line 615(659): remove one “recently”; line 625(669): “proven”; line 638(682): “chemoattractants”; line 639(683): “remains”; line 658(703): “research”
Reply: Thanks very much for pointing out this. We have corrected these typos as follows:
Line 72: “To achieve an informative summary of recent research advances”;
Line 90(88): “These collecting vessels will then meet on larger afferent lymphatic vessels opening into the draining lymph nodes (LNs)”;
Line 102:“but this needs a more in-depth digging into the phenotype change of LECs under different circumstances. ”
Line 104(106):“and is lined by LEC layers on both sides.”
Line 161(171):“The above again emphasizes the central role of Prox-1 in determining LEC differentiation and lymphangiogenesis.”
Line 190(208)： “which may be used later for in vitro and in vivo experiments.”
Line 201(219):“(Mandriota, Jussila et al. 2001, Skobe, Hawighorst et al. 2001). VEGF-C, together with the relatively less potent ligand VEGF-D (Mäkinen, Veikkola et al. 2001, Stacker, Caesar et al. 2001),”
Ling 236 (260):“HGF from fibroblasts can exert its lymphangiogenic effect”

Line 240(264):“Its stimulation of PDGFR leads to”
Line 257(280):“activating the PI3K/Akt and ERK1/2-dependent pathways in LECs”
Line 265(289):“correlation between inflammation and carcinogenesis”
Line 285(311):“integrins expressed on LECs,”
Line 286(312):“are necessary for LEC anchorage and tumor lymphangiogenesis”
Line 301(327): “and negatively regulated their proliferation”
Line 310(336):“may occur afterwards”
Line 318(344):“The association between lymphangiogenesis and tumor metastasis is shown for NSCLC and melanoma”
Line 355(380): “VEGF-C expression by tumor cells can be found to be significantly increased”
Line 385(414):“Evidence has indeed shown that”
Line 417(445): “resulting in significantly decreased LN metastasis.”
Line 501(533):“Research showed that S1P produced by Sphk in LN”
Line 584(625):“Though the therapeutic effect of anti-neovascularization has been revealed in the clinic”
Line 585(626):“therapies aiming at tumor lymphangiogenesis have gained attention only in recent years”
Line 615(659)： “Recently, the concept of tumor vessel normalization has gained attention”
Line 625(669):“Though it has been proven from clinical studies”
Line 638(682)： “whether there exist other types of chemoattractants”
Line 639(683)： “remains to be answered”
Line 658(703)： “more research is needed”

Reviewer C

Comment 1:1) The title says this is a review of recent advances but the majority of references are at least 5-10 years old. Several new developments are completely ignored. There is no mentioning, for instance, of visual evidence showing dissemination of tumor cells through the blood vessels of the lymph nodes (PMIDs 29567713, 29567714) and the role of adult lymphatic endothelial progenitors. Generation of lymphatic endothelial lineage is described only for formation of mouse embryonic vasculature but not in adult organisms, which is much more relevant to tumor lymphatics. There is long reciting of established facts on structure and function of the lymphatic system in general; it can be shortened for the current review on recent advances.

Reply: Thanks very much for pointing out this. We have added recent reference for in the manuscript for supporting the statements. We have also added discussion of dissemination of tumor cells through blood vessels of lymph nodes and the role of adult lymphatic endothelial progenitors, which is shown as follows.

Line 149: Recent evidence from murine models also showed that tumor cells may also spread through blood vessels of LNs rather than by efferent lymphatics (Brown, Assen et al. 2018, Pereira, Kedrin et al. 2018). This mode suggested that tumor cells in invaded LNs can disseminate to distant organs without the need of drainage into thoracic ducts. However, whether this form of tumor dissemination beyond LNs occur in human patients still needs to be determined(Achen and Stacker

2018).

We have also added discussion of generation of lymphatic endothelial lineage, which is shown as follows.

Line 177: In recent years, evidence showed that generation of lymphatic endothelial lineage in adult organisms, such as tumor lymphangiogenesis, may more likely take on a myloid origin (Ran and Volk-Draper 2020). This population of myloid lymphatic endothelial cell progenitors (M-LECP) derive from bone marrow hematopoietic progenitor cells that can co-express myloid and LEC markers(Gutierrez-Miranda and Yaniv 2020). During tumorigenesis, M-LECPs can differentiate in bone marrow and be recruited from blood circulation to be integrated into tumor-associated lymphatic vessels. From murine model of human cancer, it was also shown that the density of M-LECPs can be associated with LN metastasis (Volk-Draper, Patel et al. 2019).

We have also edited the discussion on lymphatic vessel structure, which is shown in Part 2 (page 3-4) of the manuscript.

Comment 2: 2) The role of tumor-recruited macrophages in generation of lymphatic vessels is not mentioned even once. Tumor recruited leukocytes are composed almost entirely of bone marrow derived myeloid cells, not T cells and not B cells. The latter under the best circumstances represent the minority of tumor-recruited host cells. Tumor microenvironment (TME) is shaped by bone marrow derived immature, immunosuppressive myeloid cells, not T cells and not B cells. It appears that the authors are not familiar with the commonly accepted typical composition of leukocytes recruited to tumors and unaware of the role of tumor-recruited myeloid cells on both tumor progression and generation of new lymphatics.

Reply: Thanks very much for pointing out this. We have added discussion of the role of tumor-recruited macrophages in generation of lymphatic vessels in TME, which is shown as follows.

Line 244: In addition to tumor cell-derived lymphangiogenic factors, recruited myloid cells can be an important source of VEGF-C (Ji 2012). Circulating monocytes are recruited by tumor-derived chemotactic factors, and can be switched to alternatively activated macrophages in TME (Chen, He et al. 2018). Being a key component of infiltrating myloid in tumor tissues, tumor-associated macrophages (TAMs) is shown to be involved in the onset and maintenance of tumor lymphangiogenesis. They can express large amount of VEGF-C/D, which may be triggered by cytokines, such as IL-1β and (Ji, Cao et al. 2014, Watari, Shibata et al. 2014).

Line594: In addition to producing lymphangiogenic factors, infiltrating TAMs can indeed transdifferentiate into LECs under pathogenic conditions (Ran and Volk-Draper 2020). Inhibiting TAM infiltration through blocking CSF-1 pathway efficiently reduced tumor lymphangiogenesis (Kubota, Takubo et al. 2009), and this can only partly be explained by their secretion of lymphangiogenic factors (Volk-Draper, Patel et al. 2019). This population of LEC progenitor cells, which are called M-LECP as was mentioned, are derived from bone marrow precursors of the monocyte-macrophage lineage, and characterized by the co-expression of markers for LECs, stem cells, M2-type macrophages, and myeloid-derived immunosuppressive cells (Religa, Cao et al. 2005, Van't Hull, Bron et al. 2014). They can be incorporated into LECs, acting as a direct structural contributor to the newly formed lymphatics (Ran and Montgomery 2012). This integration is shown to be mediated by the activation of integrin β1 by galectin 8(GAL8) derived from LECs(Bieniasz-Krzywiec, Martín-Pérez et al. 2019). As this integration

is only found under disease condition, adding more importance to their role in tumor lymphangiogenesis. The recruitment of M-LECP to TME may be through the CSF-1(Kubota, Takubo et al. 2009, Volk-Draper, Patel et al. 2019), VEGF-A (Cursiefen, Chen et al. 2004, Murakami, Zheng et al. 2008), CXCL12 (Cho, Koh et al. 2007) acting on their corresponding receptors.

Comment 3:3) The authors interchangeably use the terms lymphatic endothelial cells (LECs), lymphatic vessels (LVs) and lymphatic system. These three terms mean three different things and mixing them creates a great deal of confusion. LECs do not transport tumor cells "inside" them as suggested on lines 53-54(52-54) (vessels transport tumor cells), and LECs are not "present in and around the tumor nests", as stated in line 94. In fact, isolated LECs outside of vessels have not been reported. Moreover, LVs (if this is what was meant by LECs) are typically absent "in and around tumor nests" but are rather confined to periphery of the tumor. This statement is factually incorrect.

Reply: Thanks very much for pointing out this. We have corrected the use of LEC, and lymphatic system, which should cause less confusion for readers.

Line 53-54(52-54):"The lymphatic endothelial cell (LEC) is the primary cell that builds lymphatic vasculature, forming the wall of lymphatic vessels which transport the fluid and migrating cells inside."

Line 94:"Consistent with this notion, the presence of tumor lymphangiogenesis is correlated with a higher frequency of LN and distant metastasis, observed in several types of human cancers, including lung cancer(Renyi-Vamos, Tovari et al. 2005), melanoma(Dadras, Lange-Asschenfeldt et al. 2005), colorectal cancer(Doekhie, Morreau et al. 2008) and breast cancer(Zhang, Zhang et al. 2017)."

Comment 4:4) Several other statements in this manuscript are either inaccurate or factually incorrect. It is stated that smooth muscle cells guarantee the unidirectional flow (lines 86-87), but this is the function of valves while smooth muscle cells are usually absent in tumor micro-lymphatic vasculature. It is repeatedly stated that LECs have different subpopulations (e.g., line 102) but it is not supported by cited literature or descriptive evidence. Lines 167-192() describe specific markers of LECs but many of those are not accepted by the entire field, and based on the description they might be expressed in the vessels of the lymph node but not in LVs associated with primary tumor. For instance, CD73 and cavelolin-1 are expressed in multiple cell types (mesenchymal, blood endothelial) and have no specificity to LECs. The authors further suggest that different subsets of LECs can be isolated using these markers. This reviewer is extremely concerned by this suggestion as these markers have no LEC specificity, and cell isolation based on CD73 and other mentioned proteins would pull out a poorly defined mixture not suitable for cell type specific characterization.

Reply: Thanks very much for pointing out this. We have corrected the discussion of smooth muscle cells, which is shown as follows.

Line 86-87:"Moreover, smooth muscles surround the collecting lymphatic vessel wall to increase its flexibility."

Line 102:"Thus, defining the functioning LECs may be a better approach, but this needs a more in-depth digging into the phenotype change of LECs under different circumstances."

We have also added reference on the subpopulations of LECs, which is shown as follows.
Line 692：“Thus, it also suggests that several distinct subpopulations of LECs exist, which may fulfill diverse types of functions. The recent advance of single-cell RNA sequence indeed revealed disparate populations of LECs in LNs (Takeda, Hollmén et al. 2019), however, more research is needed as to whether subpopulations of LECs could be involved in different aspects of anti-tumor immunity and related to the sequential steps of tumor metastasis. Moreover, the detailed underlying mechanisms for the differentiation and regulation of LECs call for more exploration.”
Line 197-210: As for the discussion of LN LECs, we avoided the notion of “markers”, which is shown as below.
Moreover, besides the general LEC markers mentioned above, LECs in different LN sinuses may express distinct molecules concerning the sinus systems' specialized functions. Indeed, recent advances in single-cell sequence analysis offered great help (Takeda, Hollmén et al. 2019). It revealed that CD73 and caveolin 1 are expressed in ceiling LECs of subcapsular sinus, and tumor necrosis factor receptor superfamily member 9 (TNFRSF9) is found in the floor LECs. While macrophage receptor with collagenous structure (MARCO) is expressed in both medullary and cortical sinuses, microfibril-associated glycoprotein 4 (MFAP4) was mainly found on LECs of the capsule ceiling of the medulla and claudin 11 on LECs of lymphatic valves (Takeda, Hollmén et al. 2019).
With these identifiers, researchers are better equipped to study LECs in the tumor micro-environment. Immunostaining of LEC markers can help identify them in tissue slides. Cell sorting based on specific LEC markers can acquire relatively pure LEC populations, which may be used later for in vitro and in vivo experiments. While some molecules have clear functions, such as VEGFR-3, which is the receptor for lymphangiogenic factor VEGF-C/D, we still do not know much about the rest's functions.

Comment 5:5) It is repeatedly mentioned that lymphangiogenesis promotes tumor initiation (line 210) and a lymphangiogenic factor VEGF-C promotes growth or division of tumor cells (lines 570-571). It is not clear what the foundation for these statements is. Lymphatic vessels are generally not known to initiate tumors or affect tumor size, differentiation status or other pathological parameters besides the transport of tumor cells to lymph nodes. VEGF-C can affect only cells that express corresponding receptor(s) such as VEGFR-3 or neuropilin-2 or both. The majority of human cancers including lung carcinomas do not express these largely endothelial-specific proteins. The authors must exercise more caution in citing these reports.
Reply: Thanks very much for pointing out this. We have corrected the discussion on the roles of lymphangiogenesis and lymphangiogenic factors in cancer, which is shown as follows. We are sorry for the confusion that the original statements may cause.
Line 210 (225)：However, one report showed that relationship between VEGF-C and tumor lymphangiogenesis is only be found for early-stage melanoma, indicating that VEGF-C can induce LEC only in the beginning and that lymphangiogenesis works mainly for early dissemination of tumor(Bordry, Broggi et al. 2018).
Line 570-571(612-613)：“Indeed, miRNAs that target VEGF-C could significantly suppress lymphangiogenesis of tumor xenografts (Hu, Cheng et al. 2014).”

Comment 6:6) It is also unclear whether cytokines secreted by LECs that represent a tiny fraction of tumors have sufficient influence on the collective behavior of the entire tumor mass. The concept of LECs initiating tumors and/or playing a significant role in promoting tumor cell growth should be supported by much better description of presumably existing evidence.

Reply: Thanks very much for pointing out this. We have corrected the discussion on the role of cytokines secreted by LECs in cancer and added more supportive reference, which is shown as follows.

Similar interactions between LECs and tumor cells were also found with the CXCL9/CXCL10/CCL21-CXCR3 and CXCL12-CXCR4 axes (Müller, Homey et al. 2001, Cabioglu, Yazici et al. 2005, Kawada, Hosogi et al. 2007), showing that $CXCR3^{+}$ and $CXCR4^{+}$ cancer cells are bound with an increased propensity of LN metastasis. Moreover, concerning the association between the CXCR4 receptor and the stemness of cells, the action of LEC-produced CXCL12 through CXCR4 can induce a small population of melanoma cells with elevated expression of CD133, which acquire a cancer stem cell (CSC) phenotype and show stronger resistance to chemotherapy (Kim, Koh et al. 2010). Similarly, CCR7 can induce the functional pool of stem-like cells in breast cancer cells (Boyle, Ingman et al. 2016), though whether or not LEC-derived CCL19 and CCL21 plays a role in this change still needs to be determined. Also, CXCL5 from LECs can promote tumor cell invasion by acting on CXCR2(Lee, Jang et al. 2021). Moreover, LECs may secret mitogenic factors, such as IL-6, to directly boost the proliferation of tumor cells (Van de Velde, Ebroin et al. 2020). Taken together, by the promotion of tumor cells with stronger viability, such as the CSC population, LECs may serve to provide a cultivating environment for the highly invasive cancer cells (Karaman and Detmar 2014). However, it should also be cautious as whether these factors have sufficient influence on the collective behavior of tumor mass, and more research is needed to elucidate the role of LECs in directly promoting tumor cell growth.

Comment 7:7) This reviewer found multiple instances in which statements are supported by a wrong citation or a cited reference is not only not supportive but, in fact, directly contradictory to the authors' statement. For instance, lines 272-272 state that IL-10 decreases lymphangiogenesis via reduction of infiltrated macrophages citing reference 65. This paper says exactly an opposite: lymphangiogenesis is reduced in IL-10-/- mice and IL-10 increases lymphatic formation. Cited paper #73 is used to support the role of CD9 in providing support for lymphatic vessels. The cited paper is entirely focused on MMP-2 with no mentioning of CD9. Reference 67 is used to support some differences between inflammatory and non-inflammatory breast cancer. However, the cited paper deals with extracellular matrix and has no relation to breast cancer. Reference 78 is used to support the following statement "..Infiltrated capillary LECs with a lack of basement membrane and intercellular tight junction are more vulnerable for tumor cell to squeeze and invade (78)." The cited paper is focused entirely on the properties of LECs in the normal mouse trachea and contains no experimental evidence for lympho-vascular invasion by tumor cells.

Reply: Thanks very much for pointing out this. We have corrected the wrong use of supporting reference, which is shown as follows. We are sorry for the confusion that the misleading statements may have caused.

Line 272(293):"Moreover, the anti-inflammatory cytokines IL-10 can upregulate VEGF-C

expression from macrophage, resulting in increased lymphangiogenesis and resolution of inflammation (Hos, Bucher et al. 2016)."
Line 312-312:"In line with this, and CD9 also shows supporting function for LECs that may involve modulating the molecule organization of integrin (Iwasaki, Takeda et al. 2013) ."
Line 299-301："Interestingly, research also showed that active lymphangiogenesis presents more in inflammatory breast cancer than in non-inflammatory breast cancers (Van der Auwera, Van Laere et al. 2004, Van der Auwera, Van den Eynden et al. 2005)."
Reference 78：We deplete the reference together with the misleading statement.

Comment 8: 8) In lines 94-96, it is stated that "the presence of LECs in and around the tumor nests is correlated with a higher frequency of LN and distant metastasis, observed in several types of human cancers, including lung cancer(2), melanoma(3), colorectal cancer(4) and breast cancer(5)." The cited paper #5 reports a study that was entirely done in mice using human xenografts. There is no evidence in this paper regarding human clinical breast cancers, or presence of "LECs in and around the tumor nests" (as mentioned above, this particular statement is not supported by evidence from clinical cancers) or any kind of correlations between lymphatic vessel density and local or distant metastasis in clinical tumors.
Reply: Thanks very much for pointing out this. We have corrected the wrong use of supporting reference, and the notion of "LECs in and around the tumor nests", which is shown as follows. Consistent with this notion, the presence of tumor lymphangiogenesis is correlated with a higher frequency of LN and distant metastasis, observed in several types of human cancers, including lung cancer(Renyi-Vamos, Tovari et al. 2005), melanoma(Dadras, Lange-Asschenfeldt et al. 2005), colorectal cancer(Doekhie, Morreau et al. 2008) and breast cancer(Zhang, Zhang et al. 2017).

Comment 9: 9) All figures require modifications as well as a descriptive legend in addition to a list of abbreviations.
9A) Fig. 1 presents somewhat weird illustration of the tumor in which large circular vessels are located throughout the tumor at both the center and the periphery. There are several things that are wrong with this picture (e.g., the ratio between tumor size and node size, and the equal number 1 of afferent and efferent vessels); however, the main point is: typically, both human and mouse tumors have no lymphatic vessels anywhere farther than 400-500 microns from the tumor-host interphase, and often tumors do not have any large circular LVs at all.
Reply: Thanks very much for pointing out this. We have corrected the figure and provided figure legends together with a list of abbreviations, which is shown as follows.

Figure 1.

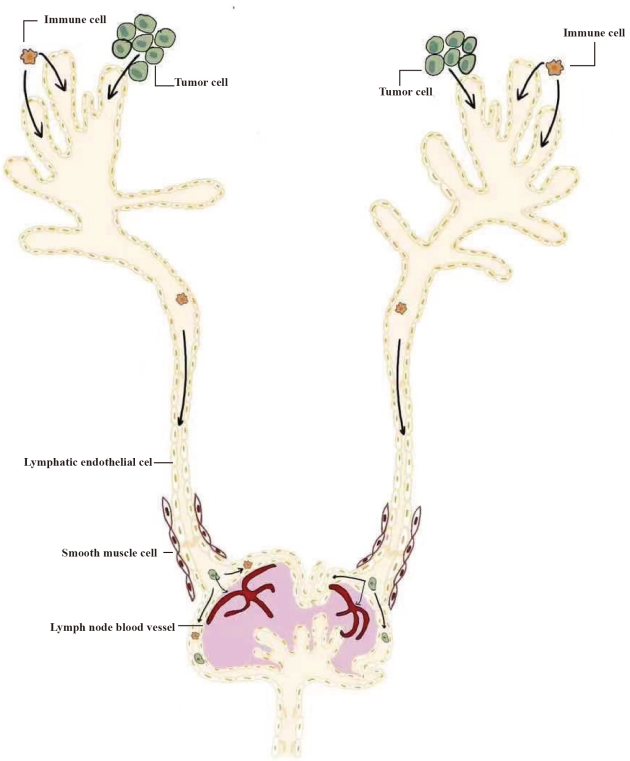

Figure 1. Sequential steps of tumor lymphatic metastasis. Sequential steps are followed during tumor lymphatic metastasis, from a primary tumor site to collecting lymphatic vessel and the draining lymph nodes. They can also invade lymph node blood vessels to colonize distant organs.

9B) Fig. 2 ignores any proportion regarding sizes of different cells in the TME. LEC is 10-fold larger than a tumor cell which is about the same size as a macrophage and a lymphocyte.
Reply: Thanks very much for pointing out this. We have corrected the figure and provided figure legends together with a list of abbreviations, which is shown as follows.

Figure 2.

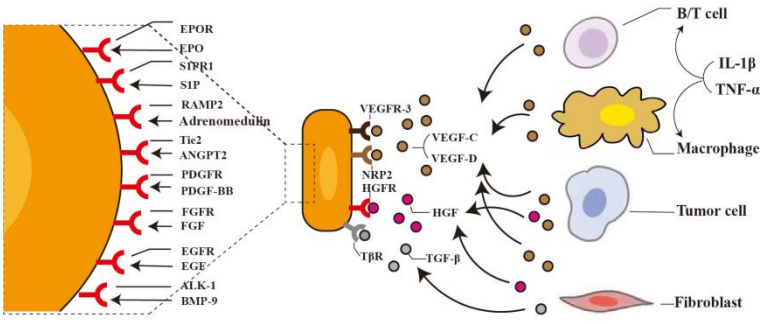

Figure 2. Lymphangiogenic and anti-lymphangiogenic factors in tumor-environment. Molecules that may influence lymphangiogenesis are shown, with their corresponding receptors. For a better view of the receptors, a magnification of LEC is shown on the left. LEC, lymphatic endothelial cell; VEGF, vascular endothelial growth factor; VEGFR ,VEGF receptor;

TNF-α , tumor necrosis factor; EPO, erythropoietin; EPOR, EPO receptor; S1P, Sphingosine-1-phosphate; S1PR1,Sphingosine 1-phosphate type 1 receptor; RAMP2, receptor activity modifying protein 2; ANGPT, angiopoietins; Tie2, ANGPT receptor; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; FGF, fibroblast growth factor; FGFR, FGF receptor; EGF, epidermal growth factor; EGFR, EGF receptor; HGF, hepatocyte growth factor; HGFR, HFG receptor; TGF-β, transforming growth factor β; TβR, TGF-β receptor; NRP-2, neuropilin-2; BMP-9, bone morphogenetic protein 9; ALK-1, activin receptor-like kinase 1.

9C) Fig. 3 has the same problem related to cell size and much more. There is now an additional cell that looks like osteoclast with 5 protrusions, but it is unlabeled and appears in three different colors (blue, purple and pink). I do not know what this cell represents and what different colors signify. CCL19 and CCL21 are shown to be produced by fibroblasts. The authors should provide some citation supporting this. Interaction between LEC and unknown cell with protrusions is shown by complex of Mac-1 on the LEC surface and ICAM-1 expressed on the other cell. Mac-1 (CD11b/CD18) is expressed primarily on myeloid cells and not on endothelial cells. ICAM-1 can be expressed in both cell types but for this interaction to occur it has to be on the LEC surface and not on the other cell. Lastly, the figure is supposed to show all possible interactions of LEC lining the vessels with different cells in TME. But, as in the text, there are no macrophages or blood vascular endothelial cells, or recruited progenitors. Presented picture is therefore incomplete. The authors bear the responsibility for producing factually accurate illustrations, particularly if their intent is to educate broad audience.

Reply: Thanks very much for pointing out this. We have corrected the figure, deplete misleading parts without solid supportive evidence and provided figure legends together with a list of abbreviations, which is shown as follows.

Figure 3.

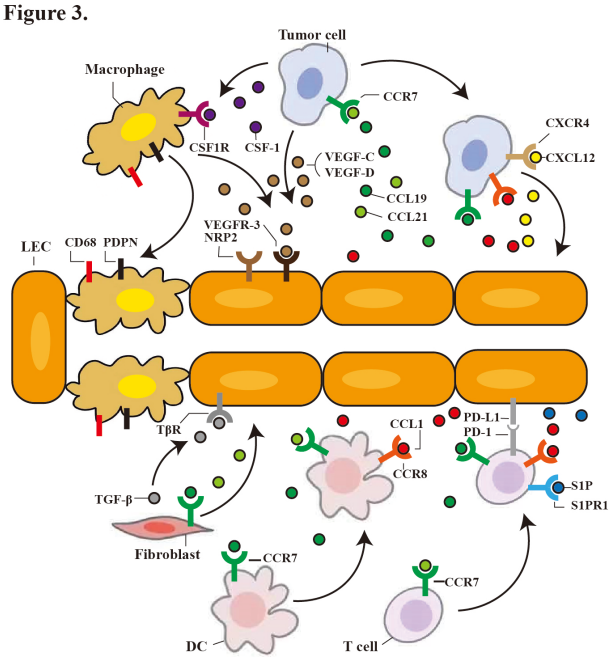

Figure 3. Interaction between lymphatic endothelial cells and tumor micro-environment. LECs can secret chemokines to recruit tumor cells, immune cells and fibroblasts. They can also affect tumor cell survival and immune cell function. Macrophages can be recruited and

integrated into lymphatic vessels. CCL1, CC-chemokine ligand 1; CCL19, CC-chemokine ligand 19; CCL21, CC-chemokine ligand 21; CXCL12, CX-chemokine ligand 12; CCR7, CC-chemokine receptor 7; CCR8, CC-chemokine receptor 8;CXCR4, CXC-chemokine receptor 4; VEGF, vascular endothelial growth factor; VEGFR ,VEGF receptor; NRP-2, neuropilin-2; TGF-β, transforming growth factor β; TβR, TGF-β receptor; S1P, Sphingosine-1-phosphate;S1PR1,Sphingosine 1-phosphate type 1 receptor; PD-1, programmed cell death-1; PD-L1, PD-1 ligand 1; DC, Dendritic cells; PDPN, podoplanin. CSF-1, colony-stimulating factor 1; CSF1R, CSF-1 receptor.

Minor critiques:

Comment 10:) The manuscript is poorly written with many statements being incomprehensible, at least to this reviewer. Many statements are written in the future tense (e.g., “smooth muscles will surround the vessel wall” on p. 3; “tumor cells will transport..” on p. 4 (and, of course, tumor cells do not transport anything, lymphatic vessels transport tumor cells); “tumor cells will reside”, p. 4), etc. These events either happen or not, based on evidentiary support; the future tense is not applicable here.

Reply: Thanks very much for pointing out this. We have corrected the incomprehensible statements, which are shown as follows.

p.3 “Moreover, smooth muscles surround the collecting lymphatic vessel wall to increase its flexibility.”

p.4 “Secondly, invaded tumor cells migrate within lymphatic vessels towards lymph nodes.”

p.4 “Thirdly, tumor cells reside and grow in the metastatic LNs”

Line 82: These together can facilitate uptake of fluid, molecules, and migrating cells from peripheral tissues.

Line 147: With the thoracic duct connecting lymphatic and blood vessels, tumor cells then spread to systematic circulation and colonize distant organs.

Line 168: The above changes can also lead to increased lymphangiogenesis.

Line 222: The downstream signaling then leads to increased survival, growth, and migratory ability of LECs.

Line 275: Moreover, EPO, which is often raised due to anemia caused by cancer therapy, increases migration, capillary-like tube formation, and dose and time-dependent LEC proliferation.

Line 319: as the membrane type 1-MMP (MT1-MMP) is found to degrade LYVE-1 on LECs, which then leads to inhibition of LYVE-1-mediated lymphangiogenic responses.

Line 329: This leads to reprogramming of BEC to LEC and reduced tumor lymphangiogenesis in a mouse model of breast cancer.

Line 402: This may imply that, while LECs facilitate the recruitment of immune cells, the local tumor micro-environment educates them to be either immunogenic or tolerant.

Line 409: the growth pressure selects those that can take the best advantage of the tumor micro-environment

Line 441: Blocking CCR8 leads to the arrest of tumor cells in collecting lymphatics at junctions where they meet subcapsular sinuses

Line 512: The secreted CCL21 then interacts with its receptor CCR7 expressed on the surface

of DCs to facilitate their entry into the lymphatic vessels
Line 534: Activated lymphocytes then transverse from LN parenchyma to medullary sinus, which then follow the flow of lymphatic fluid to efferent lymphatics and later back to the circulatory system through connection by thoracic duct.
Line 563: This cross-presentation leads to apoptosis and dysfunction of tumor-antigen-specific T cell and failure of anti-tumor immunity
Line 573: These factors can then provide the antiapoptotic and proliferative signals for T cell activation
Line 579: Much in an indirect way, the LT-LTβR interaction between B cells and fibroblastic reticular cells (FRCs) can lead to increased production of lymphangiogenic factors in B cells caused by increased B-cell-activating factor (BAFF) from FRCs.

Comment 11 :) In interest of space, I will not go through all grammatical and stylistic mistakes, but I strongly recommend substantial editing of the manuscript. A particular attention should be paid to statements like "mutual interaction between tumor cells and tumor micro-environment is more likely than a mutual interaction between tumor cells and tumor microenvironment, such as the LECs" (p. 4), or "tumor cells need to travel across the LECs for another time at the medullary sinus boundary.." on p. 3 (when did they cross LVs the first time?), or "Similar to founding with BECs, Notch signaling inhibition works in synergy with VEGF.. on p.5. Inhibition does not work in synergy with anything, and I don't know what "founding with BECs" means. These sentences make no sense.
Reply: Thanks very much for pointing out this. We have corrected the grammatical and stylistic mistakes, which are shown as follows.
p.4 "While the classical view of lymphatic metastasis is a passive transport of tumor cells under lymphatic fluid pressure, it is now confirmed that lymphatic metastasis is more likely the result of a mutual interaction between tumor cells and tumor microenvironment, such as the LECs."
p.3 "In this sense, efferent leukocytes need to travel across the LECs at the medullary sinus boundary."
p.5 "Similar to its effect on BECs, Notch signaling inhibition can work to induce LEC sprouting."

Comment 12:) The authors should replace the words "proof" or "proved" with "shown or demonstrated". Biologists do not prove anything. Presented evidence is usually indirect, subject to interpretations, and therefore, debatable.
Reply: Thanks very much for pointing out this. We have avoided the use of such words, and replace them with "show" or "shown", which is shown as follows.
Line 96: Through the staining of key markers, LECs is shown to be an integral part of them in the tumor micro-environment.
Line 619: Through immunochemistry staining, VEGFR-3-positive endothelial cell density is shown to be a significant prognostic factor in NSCLC
Line 621: Indeed, the co-expression of other lymphangiogenic factors, including FGF2 and PDGF-B, and VEGFR-3, is also shown to be strongly associated with poor survival in NSCLC patients

Comment 13:) The authors mention that bevacizumab (anti-VEGF antibody) is a success evidenced by "efficient inhibition of tumor neovascularization". This is puzzling that the authors are not aware that all clinical trials using bevacizumab as a single agent failed, and inhibition of tumor vasculature was shown only in mice. In fact, this opened a new direction in the angiogenesis field focused on understanding the underlying mechanisms of clinical failure of this promising agent in mice. The statements related to bevacizumab must be edited to reflect the reality. The authors should also re-consider their proposal to follow the same strategy for VEGF-C/D not only because anti-VEGF-A targeting clearly failed in clinics but also because anti-VEGF-C strategy has been already attempted with no evidence for success.

Reply: Thanks very much for pointing out this. We have corrected the discussion on the use of bevacizumab and anti-VEGF-C strategy in clinics, which are shown as follows.

With the approval of clinical use of monoclonal antibody (mAb) Bevacizumab that neutralizes VEGF-A for efficient inhibition of tumor neovascularization, mAbs that can target VEGF-C/D or the extracellular domain of VEGFR-3 have also been designed (Achen, Roufail et al. 2000, Persaud, Tille et al. 2004, Goldman, Rutkowski et al. 2007, Hajrasouliha, Funaki et al. 2012, Kashima, Watanabe et al. 2012, Saif, Knost et al. 2016). However, these are still in their preclinical stage, with further work to be done before clinical use. Moreover, concerning the failure of clinical trials that use bevacizumab as a single agent, the proposal of anti-VEGF-C therapy should gain more caution, and the combined therapy with other drugs directly targeting tumor cells may bear a better chance of success.

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