Cross-talk between lymphangiogenesis and malignant melanoma cells: New opinions on tumour drainage and immunization (Review)

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Abstract. Malignant melanoma (MM) is a highly aggressive tumour that can easily metastasize through the lymphatic system at the early stages. Lymph node (LN) involvement and lymphatic vessel (LV) density (LVD) represent a harbinger of an adverse prognosis, indicating a strong link between the state of the lymphatic system and the advancement of MM. Permeable capillary lymphatic vessels are the optimal conduits for melanoma cell (MMC) invasion, and lymphatic endothelial cells (LECs) can also release a variety of chemokines that actively attract MMCs expressing chemokine ligands through a gradient orientation. Moreover, due to the lower oxidative stress environment in the lymph compared with the blood circulation, MMCs are more likely to survive and colonize. The number of LVs surrounding MM is associated with tumour-infiltrating lymphocytes (TILs), which is crucial for the effectiveness of immunotherapy. On the other hand, MMCs can release various endothelial growth factors such as VEGF-C/D-VEGFR3 to mediate LN education and promote lymphangiogenesis. Tumour-derived extracellular vesicles are also used to promote lymphangiogenesis and create a microenvironment that is more conducive to tumour progression. MM is surrounded by a large number of lymphocytes. However, both LECs and MMCs are highly plastic, playing multiple roles in evading immune surveillance. They achieve this by expressing inhibitory ligands or reducing antigen recognition. In recent years, tertiary lymphoid structures have been shown to be associated with response to anti-immune checkpoint therapy, which is often a positive prognostic feature in MM. The present review discusses the interaction between lymphangiogenesis and MM metastasis, and it was concluded that the relationship between LVD and TILs and patient prognosis is analogous to a dynamically tilted scale.

Contents

1. Introduction

Malignant melanoma (MM) is caused by the transformation of pigment-producing melanocytes into cancer cells. It commonly develops in the basal layer of the skin and mucous membranes, but it can also occur in the uvea, meninges, gastrointestinal tract, and other tissues (1). The incidence of MM has continued to rise worldwide, and the 5-year survival rate is only 25% (2-4). Major treatment options include surgical resection, immunotherapy, and targeted therapy (4,5). Since MM is prone to lymphatic metastasis at the early stages, the 12-month progression-free survival rate after targeted therapy is ~35%, and the median overall survival is 23 months (6). Therefore, the remission time and the overall survival rate do not demonstrate significant improvement (5-8). Lymphatic vessel density (LVD) in patients with MM was positively associated with a poor

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prognosis (9,10). This means that when the number of lymph vessels (LVs) adjacent to the tumour is higher, the probability of tumour metastasis is higher. At the same time, LVD is also associated with tumour-infiltrating lymphocytes (TILs), which have predictive value for therapy. When the number of paracancerous LVs increases, the number of TILs in the tissue also increases. The increased TIL number could have a better response to immunotherapy in MM research, which is beneficial to the prognosis of patients (6,7). Therefore, lymphatic endothelial cells (LECs) not only serve as a suitable conduit for metastasis but also play a multifaceted role in the metastasis of MM and the host immune response. In the present review, the interactions between melanoma cells (MMCs) and LECs during MM metastasis were discussed.

2. Features of tumour lymphatic drainage

The lymphatic system is a unidirectional circulatory network involved in maintaining tissue fluid balance, absorbing lipids and conducting immune surveillance (11). Capillary lymphatic vessels (CLVs) of blind-ended origin are permeable, with only a loosely single endothelial layer (12,13). Thin fibrous structures attached to the surfaces of LECs on CLVs can sense interstitial pressure, further increasing the permeability of CLVs in inflammatory or tumour situations (11). All of the aforementioned features make nascent CLVs the best channel for tumour cells to invade. In addition, a high endothelial venules (HEVs) of the lymph node (LN) also serves as a convenient exit for metastatic MMCs (13). In a mouse model, B16 melanoma was found to enter the blood circulation through LNs and finally metastasize to the lung (14).

It is well documented that LECs have multiple functions, including the secretion of chemokines that actively recruit cells. Single-cell sequencing of LNs confirmed the presence of six functional types of LECs mapped to specific locations, lining the floor and ceiling of the subcapsular sinuses (SCSs), medullary sinuses (MSs), and valve, expressing different chemokines (15-17). LECs of the SCSs and MSs express high levels of neutrophil chemoattractants to maintain chemokine signaling gradients (15,16). Chemokines and their ligands play crucial roles in leukocyte trafficking and are involved in the metastasis of cancer to specific organs. In MM, LECs serve as the primary source for the chemokine CCL21. This chemokine attracts $CCR7^+$ MMCs towards CCL21-expressing LECs but not blood endothelial cells (18,19). As a result, it further promotes LN metastasis. Secreting CXCL12 by tumour-associated LECs at metastatic sites has been reported to attract MMCs expressing CXCR4 and promote the growth of metastases. This process can also convert tumour immunogenicity into immune tolerance, thereby promoting tumour progression (20,21). Production of the chemokine CCL1 by the lymphatic sinus within the LN mediates the entry of MMCs expressing CCR8 into the LNs. Blocking CCR8 has been shown to reduce LN metastasis (22). At the same time, high expression of the chemokines CXCL5, CXCR3 and CXCR4 has been found in a variety of melanoma experiments (23-25). Further blockade of these chemokines or their receptors with antagonists or neutralizing antibodies reduced the metastasis of MMCs (23-25).

A previous study showed that lymph from patients with MM is a rich source of tumour-derived factors including melanoma biomarkers such as LDH, S100B and S100A8, metastasis-associated proteins such as CSF-1, galectin-3, MMP-2 and MMP-9, tumor-derived factors such as IL-6, IL-8, IL-1β, IL-4, IL-10, TNF-α and extracellular vesicles. This can offer a valuable proteomic signatures in comparison to plasma contents (26). Low levels of free iron along with high levels of glutathione and oleic acid in lymph protect MMCs from oxidative stress and ferroptosis, thereby preventing subsequent metastasis (27,28). Compared with the highly oxidized state in the blood, the lymphatic circulation provides a more suitable environment for the survival and colonization of MMCs (27). When MMCs were injected into mice, the efficiency of tumour cell metastasis following intranodal injection was notably higher compared with that following intravenous injection (27). The efficiency of intravenous metastasis also increased after MMCs had been disposed of in lymph. The lymphatic system, to some extent, provides a favorable environment for tumour metastasis.

3. MM promotes lymphangiogenesis and LN education

To facilitate invasion and metastasis, MMCs, tumour-associated macrophages (TAMs) and stromal cells in the tumour microenvironment can release multiple cytokines that promote the proliferation of LECs and induce lymphangiogenesis (29-31). There is a large number of micro-LVs in MM paracancerous tissues, and quantitative studies have shown that the mean LVD in MM nests and paracancerous areas is 6.3 and 12.5 per mm2, respectively (32,33). The LVD in the paracancerous region is notably higher than that in central areas, and the LVD in metastatic MM is higher than that in primary MM (34,35). Although the link between lymphangiogenesis and metastasis has received strong support, the precise molecular mechanisms driving tumour lymphangiogenesis remain poorly understood.

VEGF-C/D-VEGFR3 is the most prominent and well-investigated signaling pathway that plays an important role in lymphangiogenesis. VEGF-C and VEGF-D are growth factors that stimulate LEC proliferation and lymphatic remodeling. These factors have been found to be upregulated in MMCs (36,37). Inhibition of lymphangiogenesis by blocking VEGFR-3 or VEGF-C/D could reduce LN colonization and distant metastasis (38,39).

In addition to VEGF family members, CXCL5 is upregulated in T4-stage MMCs, leading to a notable increase in lymphangiogenesis (25). Other factors that can also directly or indirectly promote lymphangiogenesis include angiopoietins, SRY-box transcription factor 18, fibroblast growth factor and epidermal growth factor (10,40,41).

MMCs are highly plastic and can dynamically switch phenotypes (42,43). MM promotes natural killer (NK) cell evasion and T-cell suppression by expressing major histocompatibility complex (MHC)-I and programmed death-ligand 1 (PD-L1) at high levels to facilitate LN metastasis. After LN metastasis, T-cell responses to tumours are suppressed, regulatory T cells (Tregs) are induced, and distant organ metastasis is promoted (44). Extracellular vesicles, inflammatory factors and cytokines secreted by MMCs reach the premetastatic LNs. They not only act on LECs but also cause the microenvironment

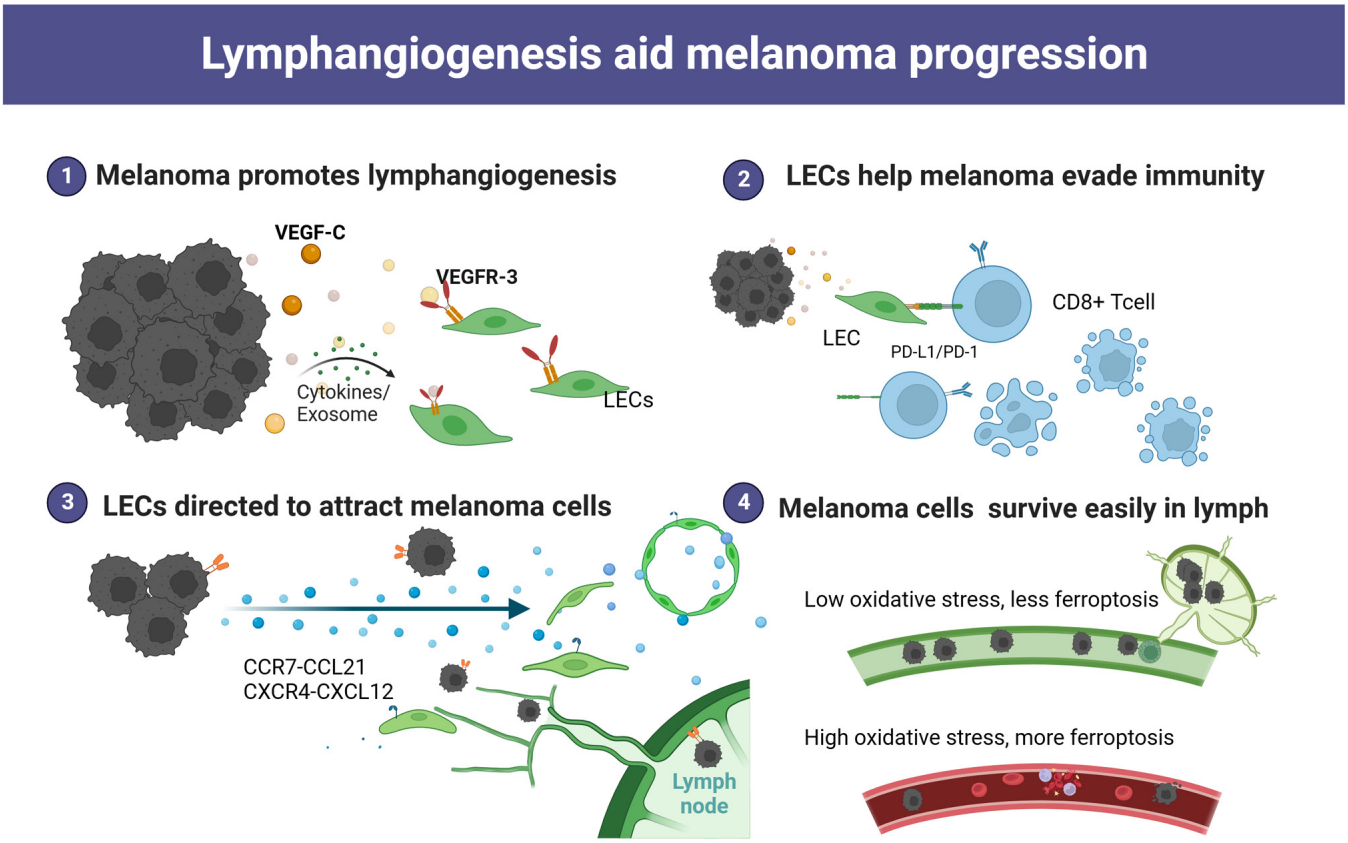

Figure 1. Melanoma promotes lymphangiogenesis. The proliferating LECs can assist melanoma to evade immunity and release chemokines to attract melanoma cells. Lymph protects melanoma cells to survive in low oxidative stress and less ferroptosis conditions. LECs, lymphatic endothelial cells; PD-1, programmed death-1; PD-L1, programmed death-ligand 1.

to be reprogrammed into a favorable niche that facilitates later colonization and supports the development of metastasis (31,45-47). Fibroblast reticular cells (FRCs) in LNs can control the LN elasticity and microarchitecture (48). Recent studies have shown that IL-1 secreted by dedifferentiated MMCs inhibits the JAK1-STAT3 and YAP pathways, which drive actomyosin contraction in FRCs (48,49). This inhibition leads to LN swelling and promotes tumour spreading in the premetastatic LN niche.

4. Role of LECs in immunomodulation

Under physiological conditions, LVs control immune cell trafficking and initiate the immune response to inhibit tumour progression. However, tumour-associated LECs display a remarkable degree of phenotypic plasticity that regulates immunotolerance (Fig. 1). LECs isolated from highly metastatic tumours showed a unique expression profile and transcriptional program compared with those from non-tumour tissues (50). Characterization of the tumour-associated LEC secretome by RNA sequencing and cytokine array revealed that IL6 is one of the most markedly regulated molecules in promoting primary tumour growth, and its production is negligible in unexposed LECs (51).

In several MM models, LECs maintain peripheral tolerance by directly upregulating PD-L1, which interacts with PD-1 on T cells to inhibit autoreactive T cells (52). The observed upregulation of PD-L1 on LECs may be stimulated by IFN-γ released in the tumour microenvironment (52,53). LECs are capable of scavenging and cross-presenting tumour-associated antigens on MHC-I, which in turn causes dysfunctional activation of $CD8^+$ T cells (54). It was shown that LECs in LN could cross-present the exogenous tumour antigen OVA to $CD8^+$ T cells, and naive LECs scavenge and cross-present OVA *in vitro*, leading to loss of function in a B16F10 melanoma model (55). In other aspects, LECs play an immunosuppressive role by dampening dendritic cell (DC) maturation, thereby reducing the ability of DCs to activate effector T cells (56,57). LECs upregulate the expression of MHC-II in human melanoma specimens and depend on IFN-γ to promote Treg proliferation and exert immunosuppressive effects in the tumour microenvironment (54,58-60). All results suggest that LECs could play a critical role in developing an immunosuppressive environment.

5. High immunogenicity and immune escape of MMCs

MM is an immunogenic malignancy, and there have been attempts to exploit this specificity to develop novel therapeutic strategies. A study found a notable number of immune cells, including different subsets of T cells, DCs, lymphocytes, macrophages, neutrophils and other cells, infiltrating around the MM tissue. This infiltration may be attributed to tumour-host interactions (61). There is a growing interest in the antitumor immune response exerted by tumour-infiltrating immune cells (TIICs) (62-64). TIIC

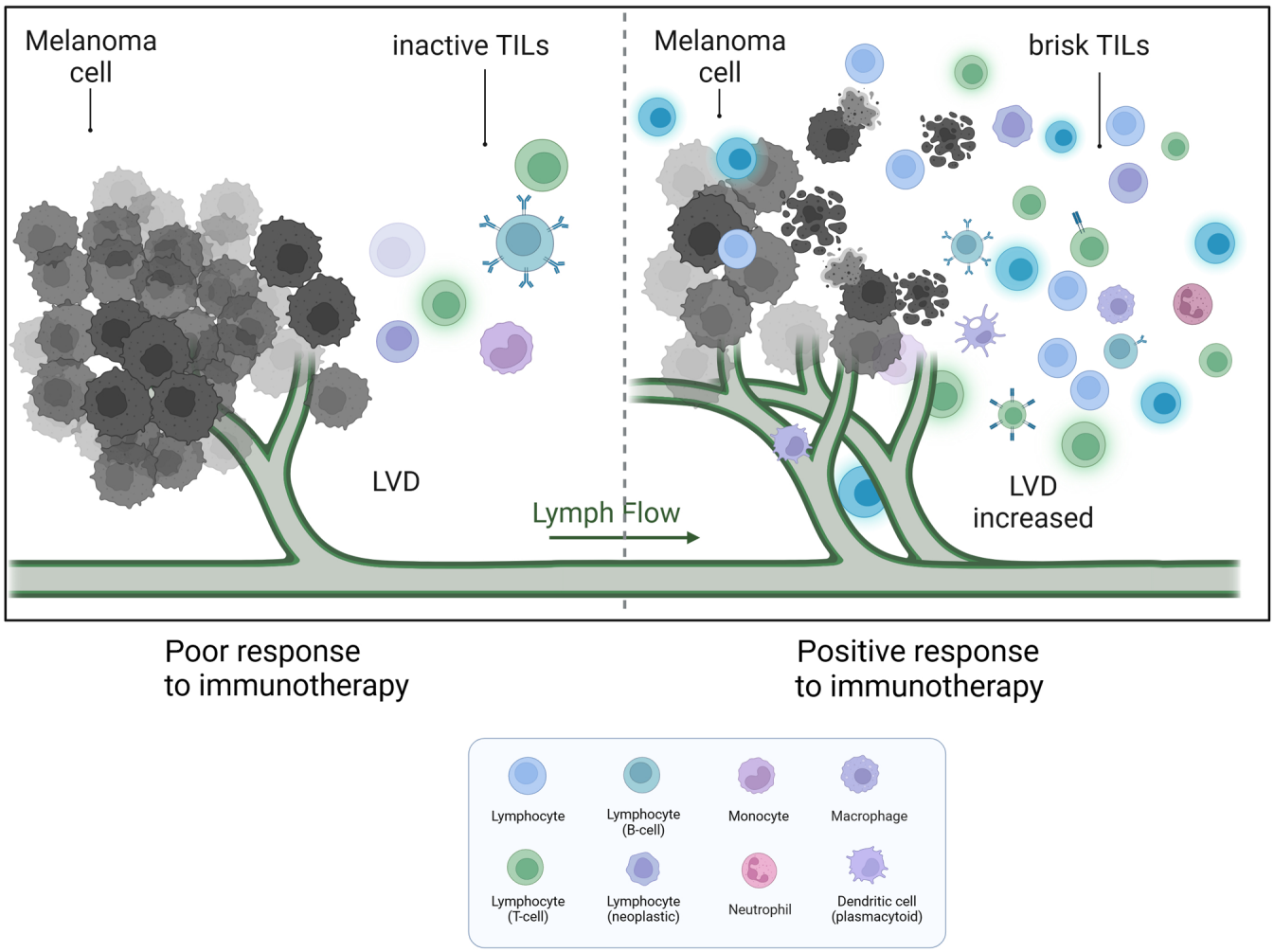

Figure 2. Increased LVD around melanoma has a relationship with the activity of TILs, and brisk lymphocytes make melanoma more sensitive to targeted therapy and immunotherapy. TILs, tumor-infiltrating lymphocytes; LVD, lymphatic vessel density.

proportion notably varies among different individuals. As a result, a certain percentage of patients with melanoma do not respond to checkpoint immunotherapy (Fig. 2). A study cohort of 2,624 patients with cutaneous melanoma found that TILs were an important histopathological characteristic reflecting host immune response. A total of 16.5% of patients had no TILs, 73.0% had inactive TILs and 10.4% had active TILs. The 5-year survival rate was 71.0% among patients without TILs and 85.2% among patients with brisk TILs. Brisk TILs were notably associated with improved overall survival (OS) (65). The presence of various subpopulations of TIICs has also been reported to predict patient response to immune checkpoint therapy.

MMCs manipulate their heterogeneity and plasticity in some recurrent cases, leading to the loss of expression of multiple tumour antigens or complete loss of HLA class I expression which allows them to evade functional antigen-specific immune recognition (66-70). The effectiveness of T-cell cytotoxicity requires proper antigen presentation by DCs. Insufficiently presented antigens cannot activate T cells and induce immunological ignorance. Altered expression of MHC-I is frequently observed on MMCs, which allows them to evade recognition by NK cells and reduces their cytolytic activity (70). Mediators including IL-8, IL-10, TGF-1 and VEGF released by MMCs or TAMs limit the maturation of normal DCs and, as a result, hinder their ability to present and activate $CD8^+$ T cells (70,71).

Activation of negative immune checkpoint molecules on MMCs shields them from immune attacks and enables further proliferation. PD-L1 is expressed at high levels on MMCs (72), and the combination of PD-1 and PD-L1 can initiate $CD8^+$ T-cell apoptosis and stimulate the differentiation of $CD4^+$ T cells into Tregs, allowing tumour cells to evade the immune system (72). $CD8^+$ T cells/Tregs in the tumour microenvironment could be used to predict the survival of patients with MM (73). Increased expression and higher affinity of cytotoxic T-lymphocyte associated protein 4 (CTLA-4) molecules on activated effector T cells in MM can prevent CD28 receptor binding to B7.1 and B7.2 on antigen presenting cells, leading to T lymphocyte deactivation (74). M2 macrophages have low antigen-presenting activity, inhibit $CD8^+$ T-cell and NK cell activity, and promote tumour cell migration (44,75). In addition, Tregs are deregulated in MM and suppress the immune system by overproducing TGF-β, IL-10 and IDO. This excessive production hampers the function of $CD4^+$ and $CD8^+$ T cells, as well as NK cells (44). MMC-derived exosomes also carry PD-L1, which can bind to T cells to suppress antitumor immunity (31,46,76).

6. Immune cell infiltration enhanced immunotherapy

Although increased LVs can promote MM spread, increased tumour-associated LECs can assist MMCs in escaping host immunity. The paradox is that tumour lymphangiogenesis promotes T-cell infiltration and potentiates immunotherapy in melanoma (77,78). There were strong positive associations between the expression levels of the lymphatic genes PDPN, LYVE1 and VEGFC, and the immune cell-specific genes CD45, CD11B, F480, KLRB1, CD3D, CD8A, CD4 and FOXP3 in human metastatic melanoma (79). Immune cell infiltration was notably reduced after MM implantation in a mouse model lacking dermal CLVs. In addition, inflammatory cytokines were markedly lower, and MM was found to be more susceptible to $CD8^+$ T-cell attack. These findings suggest that lymphangiogenesis in MM is associated with immunosuppression (80). In human metastatic melanoma, the expression of VEGF-C showed a strong association with CCL21 and T-cell inflammation. Additionally, serum concentrations of VEGF-C were found to be associated with both T-cell activation and expansion (77,80).

7. Tertiary lymphatic structures (TLS) in MM

The formation of ectopic lymphatic aggregates found in tumour or inflammatory tissues is defined as tertiary lymphoid structures (TLS). They are anatomically similar to LNs and contain T-cell areas, germinal centers with proliferating B cells, and high endothelial venules (HEVs), among others (81). TLS are typically found in the paracancerous or stromal regions rather than the core of the tumour. They also express chemokines, adhesion molecules, and integrins such as intercellular adhesion molecule (ICAM) 2, ICAM3, vascular cell adhesion molecule 1 and integrins (αL, α4, and αD), as well as CCL21, CXCL13, CCL17, CCL22 and IL-16 to facilitate lymphocyte recruitment (81). In human MM, it has been found that the presence of functional TLS activates local anti-melanoma immune responses and generally indicates a positive prognosis. The histological evaluation highlighted that metastatic melanoma contains B-cell lymphoid follicles, indicating the presence of complete TLS. By contrast, primary melanoma does not contain B-cell lymphoid follicles in TLS (82,83). Other studies have shown that TLS does occur in primary melanomas, although at a lower frequency compared with what has been reported in metastases (83-85). Using clinical samples of metastatic melanomas, it was found that B-cell markers in TLS were the most differentially expressed genes in distinguishing patients with MM with and without immunotherapeutic response, as determined by bulk RNA sequencing. Additionally, TLS also influence various T-cell phenotypes and play a crucial role in enhancing the survival of patients with MM (85,86). Therefore, the induction of B-cell-rich TLS formation to enhance the tumour response to immunotherapy can be explored as a novel strategy for treating MM.

8. Conclusion

During MM progression, MMCs reduce the expression of tumour-associated antigens to avoid their presentation and recognition. However, they also express multiple inhibitory antigens to induce immune tolerance when interacting with immune cells. MM could enhance access to tumour drainage by stimulating lymphangiogenesis through various mechanisms, which leads to increased proliferation of LECs and elevated paracancerous LVD. In the tumour microenvironment, the increased number of tumour-associated LECs recruits MMCs and immune cells, assisting MMCs in evading immune surveillance. TILs were found to have a positive association with LVD and played a role in enhancing the effectiveness of immunotherapy in MM.

In summary, increased LVD in MM promotes tumour drainage and increases TILs. While increased drainage can lead to a poor prognosis, TILs enhance the patients' response to immunotherapy and improve OS. The interaction between lymphangiogenesis and MM is complex and dynamic, and the precise mechanisms remain an open question. It may also be extended to other malignant tumours that are prone to lymphatic metastasis, such as breast cancer and squamous cell carcinoma.

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Availability of data and materials

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Authors' contributions

WJ, XHY, ZXY conceived and designed the study. WJ, HHC, WZ, DML, WZ collected information. WJ and HHC drew images and wrote the manuscript. All authors have read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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