Paradigms of vascularization in melanoma: Clinical significance and potential for therapeutic targeting

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ABSTRACT

Melanoma is the most aggressive form of skin cancer. Malignant melanoma in particular has a poor prognosis and although treatment has improved, drug resistance continues to be a challenge. Angiogenesis, the formation of blood vessels from existing microvessels, precedes the progression of melanoma from a radial growth phase to a malignant phenotype. In addition, melanoma cells can form networks of vessel-like fluid conducting channels through vasculogenic mimicry (VM). Both angiogenesis and VM have been postulated to contribute to the development of resistance to treatment and to enable metastasis. Also, the metastatic spread of melanoma is highly dependent on lymphangiogenesis, the formation of lymphatic vessels from pre-existing vessels. Interestingly, the design and clinical testing of drugs that target VM and lymphangiogenesis lag behind that of angiogenesis inhibitors. Despite this, antiangiogenic drugs have not significantly improved the overall survival of melanoma patients, thus necessitating the targeting of alternative mechanisms. In this article, I review the roles of the three paradigms of tissue perfusion, namely, angiogenesis, VM and lymphangiogenesis, in promoting melanoma progression and metastasis. This article also explores the latest development and potential opportunities in the therapeutic targeting of these processes.

1. Introduction

Melanoma is an aggressive form of skin cancer, and metastatic melanoma has a median survival rate of approximately 6 months [1,2]. Worryingly, the incidence of melanoma is increasing worldwide [3–5]. In developing countries, the increasing disease burden is exacerbated by the late detection of the cancer [5]. Novel drugs such as Vemurafenib and Dabrafenib used singly or in combination with Trametinib, a MEK inhibitor, have improved patient outcome. However, the efficacy of these treatments has been offset by disease refractoriness [1,2]. These challenges highlight the urgent need to elucidate the mechanisms involved in disease progression in order to elaborate more effective treatment approaches.

Melanoma arises from specialized pigment cells known as melanocytes, which reside in the basal epidermis [6]. In a normal physiological setting, the growth of melanocytes is regulated by secretions from keratinocytes [6]. Due to mutations in key genes that regulate cell growth, melanocytes are unable to respond appropriately to regulatory cues from keratinocytes, ultimately leading to abnormal growth [6,7]. In some cases this abnormal growth is first noticed with the formation of a nevus or mole, although melanoma can arise without a precursor lesion [7].

According to the classical model of melanoma progression, the formation of a nevus is the first stage of melanoma development [8]. According to this model, compound nevi are formed when there is an overlap in growth involving both the epidermis and dermis [1,8]. Nevi are generally benign, and can enter a radial growth phase (RGP), growing and expanding laterally, appearing as an irregular plaque [1,7,8]. Melanomas in RGP can progress to the vertical growth phase (VGP) where nests of melanocytes invade the dermis. The vertical growth phase is considered a potentially dangerous phase as the lesion in this stage has the capacity to metastasize [7]. The transition of melanocytic lesions to the VGP is promoted by angiogenesis [7,8].

Although the classical model of melanoma recognizes that nevi develop from melanocytes which have progressively gained mutations, nevi only form in approximately 26 % of lesions [1,7] Melanoma may also originate from transformed stem cells. Stem cell markers such as CD20, CD133, as well as OCT 4, NANOG and pSTAT 3 have been identified in melanoma [9,10]. The diverse cell populations with stem cell phenotypes present a challenge in melanoma therapy, since some of these cells are resistant to treatment [9]. Cancer stem cells are also known to secrete factors in response to hypoxia, promoting tumor angiogenesis, thus enabling disease progression [10]. Therefore, whatever the mechanism of melanoma development, neovessel formation
2. The link between angiogenesis and melanoma

Blood vessels can form through sprouting or intussusception, a process also known as non-sprouting angiogenesis [11]. Sprouting angiogenesis, which is referred to as angiogenesis in this review, is the de novo formation of blood vessels from an already existing microvasculature [12]. Intussusception on the other hand is the formation of new vessels through the splitting of an existing vessel [11]. Microvascular growth through intussusception in melanoma was previously described by Ribatti and colleagues, although most of the research on melanoma has focused on sprouting angiogenesis [11].

The ability of melanoma to induce angiogenesis was first observed following the transplantation of human melanoma into a hamster pouch [13]. These observations were subsequently confirmed through studies conducted by Hubler and Wolf [16], who showed neovessel formation following the transplantation of malignant melanomas into cheek pouches [14]. No vascularization was observed when keloid or normal dermal tissue was transplanted into the cheek pouches [14]. Since then, various lines of evidence have shown that there is a relationship between angiogenesis and melanoma progression. Observations from immunohistochemical analysis of human melanoma sections revealed a correlation between angiogenesis and an apparent transition from the radial to the VGP [15,16]. Tumors in the VGP were thicker, and had a higher vascular density [15].

Another study involving 113 patients revealed a gradual increase in vascularization as tumor growth progressed to the VGP [16]. Additionally, blood flow was detected in cutaneous melanomas thicker than 0.9 mm, while it was rarely detected in thinner tumors [17]. However, there was no correlation between microvascular density (MVD) and poor prognosis in melanoma patients [18,19].

Of note is that melanoma produces several factors that trigger the ‘angiogenic switch’, a change in the balance between pro- and anti-angiogenic factors causing neovascularization, some of which could be useful as prognostic markers [20–22]. One factor is produced by melanoma vascular endothelial growth factor A (VEGF-A), a member of the VEGF family and a key regulator of physiological angiogenesis [20,22]. VEGF-A binds to its cognate receptor, vascular endothelial growth factor receptor 2 (VEGFR-2), to activate nitric oxide synthase (NOS)/Src thus increasing vessel permeability and also activates the phosphotyrosinol-3 kinase/Protein kinase B (PI3k/PKB) pathway leading to endothelial cell proliferation and tube formation (Fig. 1) [20,21]. In addition to the above-mentioned consequences, VEGF-A activates focal adhesion kinase (FAK), which promotes cell migration via paxillin, further promoting angiogenesis. Increased VEGF-A levels correlate with melanoma thickness in some patients [23,24]. However, VEGF-A is not an independent predictor of poor prognosis as some studies have shown that there is no significant difference in VEGF-A levels between primary and metastatic lesions [25,26]. Another angiogenic regulator, basic fibroblast growth factor (bFGF) regulators have been identified in melanoma which may promote neovessel formation [27]. A study on 21 melanoma cell lines found that the expression of bFGF, a heparin-binding protein which stimulates angiogenesis (Fig. 1), correlated with increased vascularity in melanoma xenograft models [27]. However, in melanoma patients bFGF seems to be anecdotal [28,29].

Basic fibroblast growth factor is expressed in primary invasive melanoma as well as in metastatic lesions, but not in benign nevi [28]. In another clinical study, the presence of bFGF was not a definite predictor of poor prognosis [29], although serum bFGF levels were higher in melanoma patients when compared to healthy individuals [30]. Together with angiogenic factors such as VEGF-A, bFGF also induces the formation of vessel-like channels by aggressive melanoma cells [31]. The presence of bFGF in melanoma may signify its relevance in supporting the formation of neovessels, but given that it does not correlate with disease stage, its role in promoting disease progression may be limited.

Studies on melanoma tissue have further identified other over-expressed proangiogenic proteins including placent al growth factor (PIGF), platelet derived growth factor- AA (PDGF-AA) and platelet derived growth factor-BB (PDGF-BB) [28]. PDGFs bind to the PDGF receptor (PDGFR) and induce angiogenesis via the activation of protein kinase C (PKC) as well as p38k. Placental growth factor is known to positively regulate tumor angiogenesis [28]. Nevertheless, the prognostic value of PIGF and PDGFs in melanoma remains to be established.

Interleukin-8 (IL-8), a chemokine coded for by the CXCL8 gene and which mediates inflammation, was found to be a predictor of overall survival in melanoma patients [24,30]. By binding to the G-protein coupled receptors CXCR-1 and -2, IL-8 can activate extracellular signal-regulated kinase (ERK) 1/2, ultimately stimulating angiogenesis (Fig. 1) [25]. Interleukin-8 was found to be overexpressed in metastatic cutaneous melanoma but was not detected in the normal epidermis and in benign melanoma [32]. In a study conducted on 125 cases, elevated serum IL-8 levels were measured in patients with melanoma but not in control patients who did not have the cancer [30]. Additionally, high serum IL-8 levels correlated with advanced disease and poor prognosis [30].

Another investigation on 1 344 patients demonstrated a link between IL-8 and the progression of renal cell carcinoma (RCC), melanoma and non small cell lung carcinoma (NSCLC) [33]. High serum levels of IL-8 were correlated with a drop in overall survival (OS) across the different tumor types [33]. Conversely, low serum IL-8 levels were associated with a positive response to treatment in a cohort of melanoma patients [33]. These observations highlight the importance of IL-8 in melanoma vascularization as well as in disease progression.

Currently available angiogenesis inhibitors (AIs) have had a limited impact on malignant melanomas. In future, new approaches need to consider the inhibition of multiple angiogenic regulators detected in melanoma, and include combinations with immune therapies that target IL-8 and its receptors. Strategies should also consider that melanoma cells can form vessel-like structures through vasculogenic mimicry in order to sustain tumor progression. Some scholarly works have suggested that the induction of VM may contribute to the ineffectiveness of current antiangiogenic agents in melanoma.

3. Vasculogenic mimicry provides alternative “vasculature"

Vasculogenic mimicry (VM) is the formation of vessel-like structures lined by tumor cells [34,35]. The phenomenon was recognized as a novel paradigm of perfusion in melanoma following the observation of periodic acid Schiff (PAS) stained channels [34]. Vasculogenic mimicry was first observed in uveal melanoma in the early 1990’s, since then many studies have revealed that VM loops are restricted to highly aggressive melanomas, and do not involve normal skin [35]. Subsequent studies have further revealed that fluid flows through VM channels [35,36].

The first evidence that VM networks conducted blood came from laser scanning confocal angiography studies, which showed that fluid injected into these channels entered the circulation [35]. The channels formed via VM also appear to anastomose with endothelial cell lined microvessels [37]. In a previous study tumor cells were identified in the VM-angiogenesis junction [37]. These observations confirmed the movement of tumor cells from VM channels into a functional microvasculature, possibly contributing to metastasis.

3.1. Molecular regulators of vasculogenic mimicry

The upregulation of the genes linked to angiogenesis appears to underlie the acquisition of endothelial cell (EC) characteristics by melanoma cells [38]. One of the potent EC mitogens, VEGF-A, promotes VM through binding to the vascular endothelial growth factor receptor-1 (VEGFR-1) [38]. However, the silencing of VEGF-A does not subvert...
VM, but rather results in an adaptive response characterized by the increased expression of HIF-1α [39,40]. Another growth factor, nodal, appears to be indispensable for the formation VM. Nodal, a member of the transforming growth factor (TGF-β) superfamily, promotes features of VM in vitro and in vivo [41]. Furthermore, nodal appears to be exclusively expressed in melanomas in the VGP and in metastatic lesions [42].

In the normal physiological setting, nodal is mainly expressed in embryonic tissue [39]. However, studies have also shown the expression of nodal in tissue samples of patients with stage 4 melanoma [43]. The expression of nodal in these patients did not change after treatment with a BRAF inhibitor that was administered singly or in combination with Trametinib [43]. Dacarbazine (DTIC) resistant melanoma tissues have also been found to express nodal [44]. In vitro, anti-nodal treatment suppressed melanoma cell growth, and its effectiveness was increased when combined with DTIC [44]. The feasibility of targeting nodal was further demonstrated in a metastatic A375 SM melanoma model treated with dabrafenib and a nodal antibody. The combination treatment significantly inhibited lung metastasis [43].

The expression of nodal is regulated by a family of transmembrane receptors, namely, Notch 1–4 [45]. Pertinent to melanoma, Notch 1 promotes the VGP by activating the MAPK and PI3k/PKB pathways [45]. Interestingly, inhibiting PI3k reverses neoplastic growth associated with Notch 1 (Fig. 2) [45]. In addition to Notch 1, Notch 4, which functions mainly in vascular development, is exclusively expressed by aggressive melanomas [39]. Noteworthy is that Notch 4 has been localized to melanoma cells lining VM channels [40]. Notch 4 regulates the expression of matrix metalloproteinases (MMPs), including matrix metalloproteinases-2 (MMP-2) [46,47]. Interestingly, MMP-2 together with matrix metalloproteinase-14 (MMP-14) reportedly play critical roles in the development of VM in melanoma [48]. Microarray analysis further revealed that MMP-14 and MMP-2 were up-regulated in melanoma [49]. Increased MMP-14 activity has been associated with the cleavage of laminin 5/2 into its pro-migratory fragments, namely, γ2′ and γ2x [49]. The laminin fragments can also be formed via a pathway initiated following the activation of BRAF. Noteworthy is that the BRAF gene is mutated in 30–60 % of melanomas [1,2].

When BRAF is mutated, it leads to the activation of the MEK1/2 pathway which in turn causes the phosphorylation of extracellular receptor kinase 1/2 (ERK1/2) and the activation of phosphoinositide 3-kinase (PI3k) [11]. Thus targeting BRAF downstream of PI3k may have clinical benefit.

A transmembrane protein associated with aggressive melanoma, vascular endothelial cadherin (VE-cadherin), which co-localizes and interacts with ephrin type A receptor 2 (EphA2) also activates PI3k [50]. In turn, PI3k converts pro-membrane type1 metalloproteinase (MT1-MMP) to active MT1-MMP, also known as matrix metalloproteinase 14 (MMP14). It is worth noting that active MT1-MMP promotes VM by inducing the cleavage of laminin 5γ2 both via MMP2 and independently of this proteinase [14]. Notch and nodal promote the transcription of several molecules which regulate VM, including HIF-1α, VEGF-A, VEGFR-1, VE-cadherin and EphA2 via SMAD and NICD respectively. It is important to note that VE-cadherin, a junctional protein which mediates calcium-dependent cell–cell interactions, is overexpressed in melanoma [51]. Also, VE-cadherin has been shown to interact with EphA2 and to promote VM in aggressive melanoma [51,52].

A novel model of VM, which uses aggressive uveal and cutaneous melanoma cells cultured in 3D in a collagen matrix scaffold has provided further evidence of the angiogenic molecules that are linked to VM formation in this tumor. Gene expression data obtained from RT-PCR and microarrays revealed an upregulation of the genes EFN1-A1, EFN1-A2, and TEM1 [53]. Immunostaining revealed increased levels of the cytokines CXCL-5 and CCL11, as well as ephrins -A1, -A2 and -B2. Both CXCL-5 and CCL11 are chemoattractants for endothelial cells [53]. In addition PDGF-A was found to be overexpressed by melanoma, while VEGF-A was detected only by RT-PCR but not with microarrays [53]. Therefore, nodal, VE-cadherin/EphA2 and matrix proteins MMP-2 and -14, may have clinical relevance and warrant consideration when designing targeted therapies for melanoma.

### 3.2. Clinical significance of vasculogenic mimicry

Various studies have revealed a positive relationship between the presence of VM channels and disease progression in cancer patients, including melanoma [54,55]. Immunohistochemical analysis of tissue samples from 118 melanoma patients identified the presence of VM...
networks, especially in malignant lesions [56]. In both hepatocellular carcinoma (HCC) and melanoma, VM correlates with an increased occurrence of metastasis and poor clinical outcome [57,58]. Additionally, a 10-year follow-up study showed that increased VM was associated with malignancy in cutaneous melanoma, and that it had prognostic relevance in this neoplasm [59]. Furthermore, meta-analysis data from 15 types of malignant tumors revealed that patients with VM-positive cancers had a less favorable 5-year overall survival rate when compared to patients with VM-negative cancers, and these differences were more pronounced in advanced-stage disease [60]. The importance of VM as an indicator of disease progression is further supported by the use of VM as an independent prognostic marker for colorectal cancer [61].

Vasculogenic mimicry also affects cancer treatment because it is associated with the development of resistance to therapy [62,63]. The presence of VM channels correlates with a reduction in cancer response to etoposide and cisplatin [62]. Additionally, VM-rich regions in Merkel cell carcinoma were shown to be resistant to conventional chemotherapy [63]. Although investigators have characterized some of the features of VM, much remains to be done to elucidate the mechanisms underpinning VM and to translate the generated knowledge to treatment.

3.3. Therapeutic targeting of vasculogenic mimicry

Various antiangiogenic drugs have been tested against VM, but were mostly ineffective [9]. Bevacizumab failed to inhibit VM in preclinical studies [64]. The anti-angiogenic drug endostatin was also ineffective in inhibiting VM [65,66]. In contrast, some of the compounds designed to target molecular regulators of VM have shown promising results during preclinical testing [67,68]. One such compound, resveratrol, blocks the VEGFR-1 receptor and inhibits VM [68]. However, although resveratrol was effective in vitro, it could be ineffective in patients since the inhibition of a ligand that binds VEGFR-1, VEGF-A, was shown to be ineffective in reducing VM formation.

Doxycycline and nicotinamide inhibit VM by down-regulating VE-cadherin [69,70]. Interestingly, the down-regulation of VE-cadherin by nicotinamide was also associated with the disruption of already existing VM channels [70].

Compounds that target VE-cadherin, Ginsenoside Rg3 and vadimezan, were found to be effective in inhibiting VM and tumor growth in preclinical melanoma models [71,72]. Concerning other key regulators of VM, namely nodal and EphA2, their inhibition has been shown to reduce VM. The down-regulation of nodal is also associated with reduced tumor cell proliferation and invasion [73]. A drug which targets EphA2, SiRNA EphA2DOOC, has progressed to phase I clinical trials (Table 1) [73]. Other drugs with potential to curtail VM by targeting the various regulators of VM are in clinical development (Table 1).

These drugs may have potential application in melanoma therapy, especially given that meta-analysis data from 3,600 patients with various cancers including melanoma, has revealed a significant correlation between the presence of VM channels and lymph node metastasis [82].

Table 1

<table>
<thead>
<tr>
<th>Drug Target</th>
<th>Phase</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Rapamycin mTOR, VEGF-A</td>
<td>Preclinical [74]</td>
<td></td>
</tr>
<tr>
<td>Vadimezan MAPK, VE-cadherin</td>
<td>Preclinical [71]</td>
<td></td>
</tr>
<tr>
<td>Doxycycline Inhibition of EMT</td>
<td>Preclinical [69]</td>
<td></td>
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<tr>
<td>Apitolisib PI3K/mTOR</td>
<td>Phase II [75]</td>
<td></td>
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<tr>
<td>Demcizumab Inhibition of Notch 1, 4 signaling</td>
<td>Preclinical [76]</td>
<td></td>
</tr>
<tr>
<td>CVM-1118 RAF, PDGF, VEGF-A</td>
<td>Phase I, II (recruiting) [77]</td>
<td></td>
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<tr>
<td>Panobinostat HDAC/hypoxia</td>
<td>Approved, 2015 [78]</td>
<td></td>
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<tr>
<td>MEN-1611 PI3K</td>
<td>Phase I [79]</td>
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<tr>
<td>Copanlisib PI3K</td>
<td>Approved, 2017 [80]</td>
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<tr>
<td>Burcalisib PI3K</td>
<td>Phase III [81]</td>
<td></td>
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<tr>
<td>SiRNA EphA2DOOC EphA2</td>
<td>Phase I [73]</td>
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EMT – epithelial to mesenchymal transition.

Fig. 2. Major pathways involved in vasculogenic mimicry in melanoma. Notch/Nodal mediates the expression of important signaling molecules such as VEGF-A, VE-cadherin, and EphA2. VEGF-A/VEGFR-1, BRAF, VE-cadherin and EphA2 lead to the cleavage of laminin 5γ2 into the pro-migratory fragments 5γ2x and 5γ2', which in turn promote VM.
4. Role of the lymphatic system in melanoma metastasis

The lymph nodes, together with lymphatic vessels play an important role in fluid homeostasis as well as in immunity, but can also enable tumor cell dissemination [83,84]. The spread of melanoma to regional lymph nodes represents one of the first steps in the metastasis of the tumor and is considered to be a key prognostic indicator [28,84].

Lymphangiogenesis, the formation of lymphatic vessels from a pre-existing vasculature, is associated with increased melanoma metastasis [85,86]. The formation of lymph vessels can occur within the lesion (intra-tumorally) and in the tumor periphery (peri-tumorally) or from sentinel nodes [83]. In melanoma, there is a strong correlation between intra- and peri-tumoral lymphatic vessel density and the degree of metastasis, thus highlighting the importance of the lymphatic system in disease progression. Noteworthy is that melanoma induces lymphangiogenesis during the pre-metastatic niche, which in turn supports metastasis [85].

4.1. Molecular regulators implicated in lymphatic metastasis

Hypoxia inducible factor-1α (HIF-1α) is stimulated in response to intra-tumoral hypoxia, and in turn activates vascular endothelial growth factor-C (VEGF-C) (Fig. 3), vascular endothelial growth factor-D (VEGF-D), TGF-β and Prox-1 [87]. The activation of these proteins ultimately leads to hypoxia-induced lymphangiogenesis [87]. In addition, both VEGF-C and HIF-1α mediate the expression of the cytokine CXCR4, which stimulates lymphangiogenesis [88].

Vascular endothelial growth factor-C, a member of the VEGF family, stimulates lymphangiogenesis by binding to vascular endothelial growth factor receptor-3 (VEGFR-3) [89]. In vitro, VEGF-C is overexpressed in human melanoma cells. In melanoma-bearing transgenic mice the overexpression of VEGF-C correlates with lymphangiogenesis in sentinel lymph nodes [89,90]. Additionally, increased levels of VEGF-C have been linked to the metastatic spread of melanoma [91,92].

Importantly, primary human melanoma tissue was found to express VEGF-C mRNA, the expression of which was correlated with disease progression [93]. In another study conducted on tissue samples from 100 melanoma patients, VEGF-C expression and lymph vessel density (LVD) were predictors of metastasis [94].

Vascular endothelial growth factor-D (VEGF-D) also stimulates lymphangiogenesis, and similar to VEGF-C, exerts its effects via VEGFR-3 [95]. The ligand has been detected in melanoma tissue but not in normal skin tissue or distant vessels [95]. Thus VEGF-D may also be involved in promoting the dissemination of melanoma via the lymphatic system. Additionally, certain cytokines secreted by ECs in the tumor stroma are known to promote lymphatic metastasis [96]. Pertinent to melanoma, lymphatic endothelial cells secrete the chemokine CCL21, which binds to the G protein-coupled chemokine receptor-7 (CCR7) [97]. Of note is that CCR7 is overexpressed in murine and human melanoma tissue [97].

Experiments have further shown that when overexpressed, CCR7 is associated with the trafficking and homing of tumor cells to lymph nodes [98]. Not surprisingly, in pre-clinical models, the binding of CCL21 to CCR7 promotes the migration of melanoma cells to lymph nodes [97,98]. It is thus plausible that the CCR7−CCL21 signaling pathway promotes lymph node metastasis in melanoma patients. CCR7 signaling has also been linked to the activation and increase of VEGF-C,
and a high degree of tumor cell invasion into the lymphatic vessels [99]. The silencing of CCR7 correlates with a decrease in both VEGF-C mRNA and the VEGF-C protein, and results in the inhibition of cancer cell migration and invasion [99]. Thus, CCR7 does not only promote tumor cell migration and invasion, but also potentiates VEGF-C. This makes the CCL21 – CCR7 pathway an attractive target for the inhibition of melanoma metastasis. In addition to the CCL21 pathway, angio- poietins and the Tie2 receptor play a significant role in regulating melanoma lymphangiogenesis (99).

Angiopoietin-1 (Ang-1) is required for lymphatic endothelial cell (LEC) proliferation, vessel sprouting and enlargement, while angiopoietin-2 (Ang-2) is crucial for lymph vessel maturation and stabilization [99]. Interestingly, the levels of Ang-2 and its receptor Tie-2 were found to be increased in melanoma patients [100]. In addition, Ang-2 levels were significantly higher in patients with distant metastasis when compared to patients without metastatic disease [100]. Thus, the Ang/Tie pathway may also have therapeutic relevance in melanoma.

4.2. Therapeutic implications of targeting lymph vessels

Lymphangiogenesis represents a crucial step in enabling melanoma metastasis and contributes to treatment resistance [101]. Currently, there are no drugs on the market that target tumor lymphangiogenesis. Nevertheless, a few drugs are under development and some have been investigated in pre-clinical models while others have progressed to clinical testing [102,103]. Drug development in this context has largely focused on the most important axis in lymphangiogenesis, the VEGF-C/-D – VEGFR-3 pathway (Fig. 3).

A humanized antibody that neutralizes VEGF-C, VGX-100 (Fig. 2), was evaluated in a Phase I clinical trial for advanced or metastatic solid tumors that are refractory to standard treatments [102]. The drug was well tolerated both when administered as a single agent and in combination with bevacizumab [102]. Another antibody which targets VEGFR-3, namely IMC-3C5, was tested in patients with advanced refractory solid tumors as well as in RCC. This drug had a median progression free survival rate of 6.3 weeks [103]. For patients with RCC, the treatment was well tolerated, however, minimal anti-tumor effects were realized, and stable disease was observed in 19 % of the patients [104].

In addition, several drugs that inhibit the Ang/Tie system have been developed and some have undergone clinical testing. Trebananib is a neutralizing peptibody which binds both Ang-1 and Ang-2. The drug was found to improve quality of life; however, when tested in combination with bevacizumab, there was no clinical benefit [104]. Other drugs with potential to inhibit lymphatic metastasis that are in clinical testing are REGN910 and ARRY614 (Fig. 3) [105]. These drugs target angiopoietin-2 (Ang-2) and the Tie2 receptor respectively [105]. Given the roles of both Ang-2 and Tie2 in lymphatic vessel remodeling and stabilization, as well as their elevated levels in metastatic malignant melanocytic tumors, these drugs may hold promise as part of combination strategies in melanoma therapy.

In addition, abexinostat, a drug which suppresses HIF-1α protein expression by inhibiting histone deacetylase (HDAC) should be considered in multi-targeting approaches [106]. Phase I clinical studies have revealed that abexinostat enhances the effects of the angiogenesis inhibitor pazopanib [107]. Furthermore, the inclusion of abexinostat enabled prolonged exposure of patients to pazopanib, and the development of resistance was not observed during the course of the study [107].

5. Conclusions

Significant breakthroughs have been made in the development of effective therapies for melanoma. However, disease refractoriness continues to be a challenge, and is partly attributed to melanoma vascularization. Previously, angiogenesis was postulated to be the mode of vessel formation that enables melanoma transition to the malignant phenotype. Anti-angiogenic drugs that target VEGF-A signaling such as bevacizumab were thus employed to treat melanoma, but with poor results. This is due, in part, to the fact that in addition to VEGF-A melanoma expresses a distinct pattern of angiogenic markers, as observed from several studies involving melanoma patients and from the 3D model of VM. Additionally, attributes of VM have been identified in the premalignant phase, implying that this phenomenon may not only be triggered as a result of anti-angiogenesis therapy, but that VM channels may contribute to malignant transformation in melanoma. This observation changes the way melanoma vascularization has been viewed and warrants that strategies aimed at limiting melanoma perfusion also consider VM targeting. Noteworthy is that one of the regulators of VM, EphA is implicated in the development of resistance to BRAF and MEK inhibitors in melanoma. Therefore, drugs such as vandemezan and the photosensitizer Verteporfin which target VE-cadherin and a key enzyme in promoting VM, MMP-2, may be of clinical benefit in the treatment of melanoma. Indeed, beyond supporting tumor growth, VM also correlates with lymph node metastasis, a key prognostic indicator in melanoma.

Pertinent to the lymphogenous spread of melanoma, there have been significant milestones in characterizing key role players in this process, mainly the VEGF-C/-D/VEGFR-3 axis, the CCL21 – CCR7 pathway as well as Ang/Tie signaling. Accordingly, drugs which target these pathways such as REGN910 and ARRY614, are in clinical trials and if used with immunotherapy, these treatments may have potential to restrict lymph node metastasis in melanoma patients. As well, the inclusion of drugs such as abexinostat which curtail HIF-1α expression represents a plausible therapeutic strategy. It is evident that HIF-1α plays a pivotal role in the initiation of angiogenesis, VM and lymphangiogenesis. The therapeutic approaches aimed at overcoming resistance to therapy in melanoma could be enhanced by combination treatments targeting more than one paradigm of melanoma vascularization, as well as the future elucidation of their molecular regulators. Indeed, the success of such strategies will rely on the identification of predictive biomarkers that enable effective monitoring of treatment.

Declaration of Competing Interest

The author has declared no conflict of interest.

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References

[5] D.E. Godar, M. Subramanian, S.J. Merrill, Cutaneous malignant melanoma incidences analyzed worldwide by sex, age, and skin type on personal Ultraviolet-B dose shows no role for sunscreen but implies one for Vitamin D3, Dermatolendocrinol. 9 (2017) e1267077.
stromal HGF immunoreactivity as a biomarker for melanoma response to RAF inhibitors, Mod. Pathol. 27 (2014) 1193.


[71] Y. Liu, Doxycycline as an inhibitor of the epithelial-to-mesenchymal transition and inhibitors, Mod. Pathol. 27 (2014) 1193.


[77] Y. Liu, Doxycycline as an inhibitor of the epithelial-to-mesenchymal transition and inhibitors, Mod. Pathol. 27 (2014) 1193.


[83] Y. Liu, Doxycycline as an inhibitor of the epithelial-to-mesenchymal transition and inhibitors, Mod. Pathol. 27 (2014) 1193.
