Biomedicine & Pharmacotherapy 127 (2020) 110135

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Review

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

Peace Mabeta*

Angiogenesis Laboratory, Department of Physiology, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa

ARTICLE INFO

Keywords:
Melanoma
Metastasis
Lymphangiogenesis
Angiogenesis
Vasculogenic mimicry

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

* Corresponding author at: P/Bag X323, Angiogenesis Lab, Dept. of Physiology, Faculty of Health Sciences, University of Pretoria Pretoria, 0001, South Africa.
E-mail address: peace.mabeta@up.ac.za.

https://doi.org/10.1016/j.biopha.2020.110135
Received 26 November 2019; Received in revised form 16 March 2020; Accepted 25 March 2020

precedes tumor progression.

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 (1976), 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 phoshotidylinositol-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 t 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 placental growth factor (PlGF), 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 PI3k. Placental growth factor is known to positively regulate tumor angiogenesis [28]. Nevertheless, the prognostic value of PlGF 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

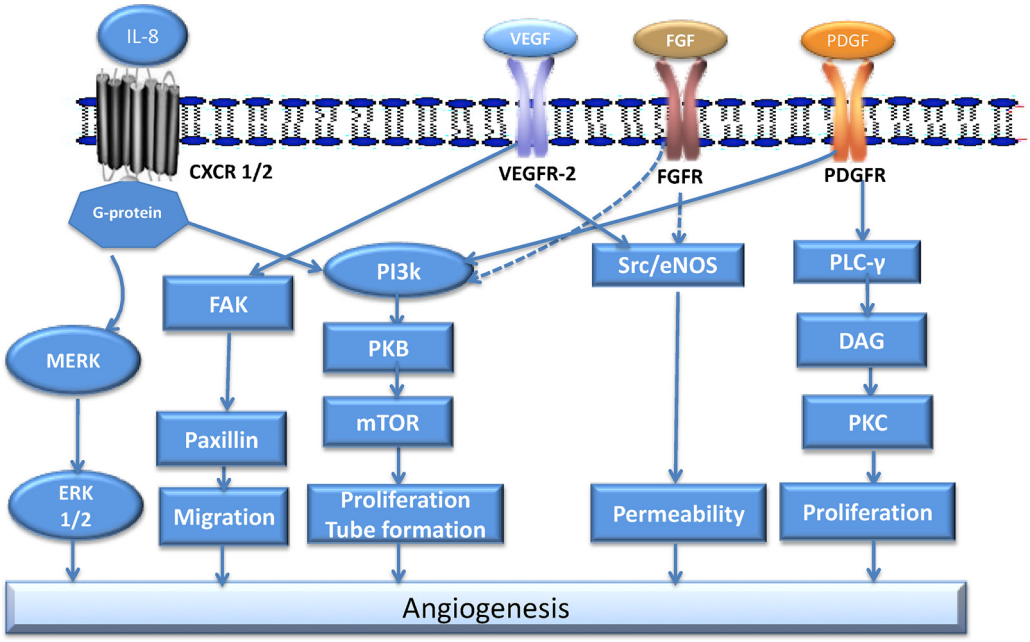

Fig. 1. Signaling pathways that contribute to angiogenesis in melanoma. Growth factors expressed in melanoma tissue have been shown to elicit pathways that promote angiogenesis. Interleukin-8, which binds G-coupled receptor proteins 1 and 2 also promotes angiogenesis via the EKR 1/2 and PI3K/PKB pathways.

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 β (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 showed 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 5γ2 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 matrix 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 EFNA1, EFNA3, EFNB2, and TIMP3 [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

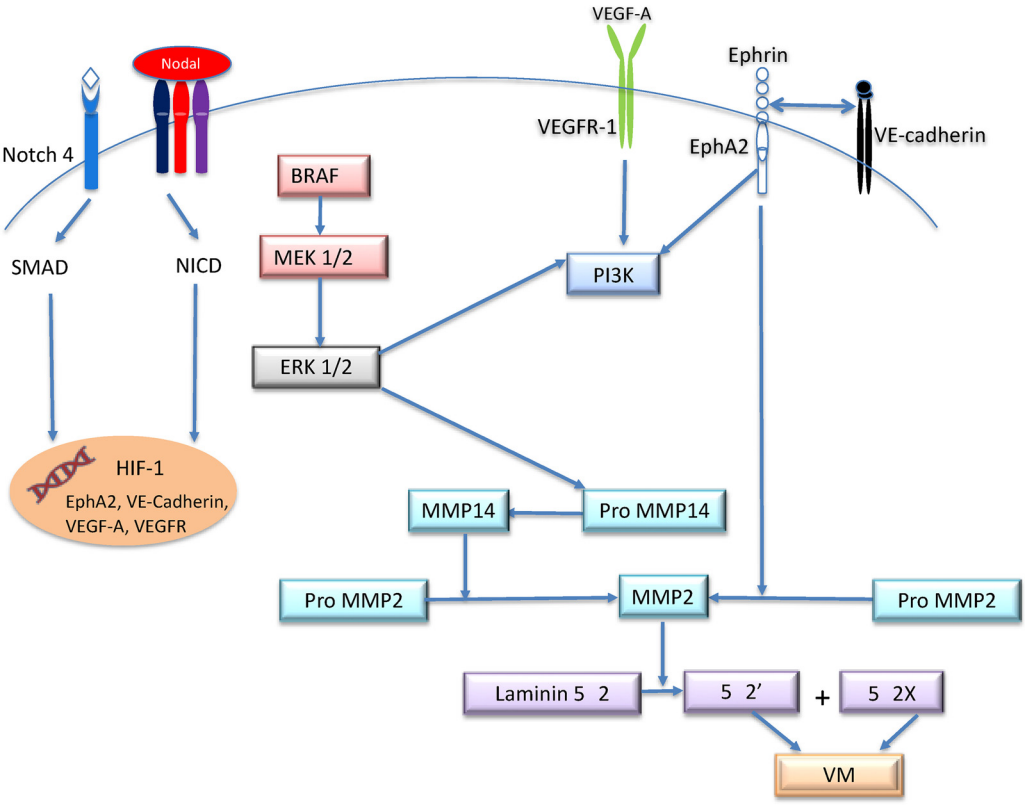

Fig. 2. Major pathways involved in vasculogenic mimicry in melanoma. Notch/Nodal mediates the expression of important signaling molecules such as VEGF-A, VE-cadherin, and EphA2. VEGF-A/VEGFR-1, BRAF, VE-cadherin and EphA2 lead to the cleavage of laminin 5γ2 into the pro-migratory fragments 5γ2x and 5γ2′,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

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 pre-clinical 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
Drugs investigated against vasculogenic mimicry in various cancers.

Drug	Target	Phase	Reference
Rapamycin	mTOR, VEGF-A	Preclinical	[74]
Vadimezan	MAPK, VE-cadherin	Preclinical	[71]
Doxycycline	Inhibition of EMT	Preclinical	[69]
Apitolisib	PI3k/mTOR	Phase II	[75]
Demcizumab	Inhibition of Notch 1, 4 signaling	Preclinical	[76]
CVM-1118	RAF, PDGF, VEGF-A	Phase I, II (recruiting)	[77]
Panobinostat	HDAC/hypoxia	Approved, 2015	[78]
MEN-1611	PI3k	Phase I	[79]
Copanlisib	PI3k	Approved, 2017	[80]
Burpalisib	PI3k	Phase III	[81]
SiRNA EphA2DOOC	EphA2	Phase I	[73]

EMT – epithelial to mesenchymal transition.

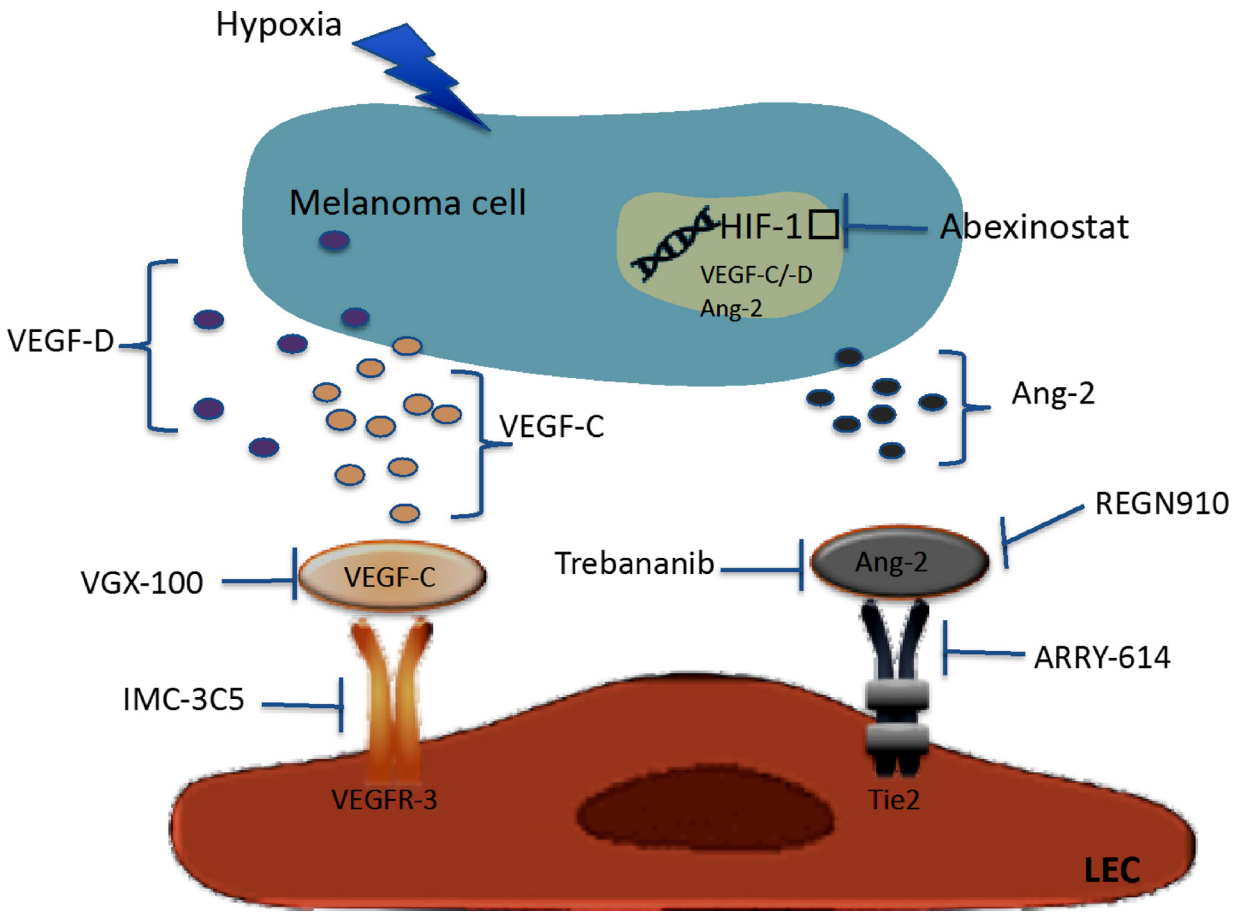

Fig. 3. Drugs with potential to block the lymphogenous spread of melanoma.
Hypoxia stimulates the expression of VEGF-C/-D, which in turn bind to VEGFR-3 to promote lymphangiogenesis. Trebananib and ARRY-614 inhibit Ang-2 and Tie-2 respectively. VGX-100 inhibits VEGF-C while IMC-3C5 blocks VEGFR-3. Abexinostat downregulates the expression of HIF-1α protein. LEC - lymphatic endothelial cell

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 over-expressed 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, angiopoietins 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 vadimezan 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 – CCR-7 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.

Acknowledgement

Funding for this work was provided by the National Research Foundation (Project 114403). Dr Cheryl Tosh (Science editor, Faculty of Health Sciences, University of Pretoria) provided editorial assistance.

References

[1] V. Gray-Schopfer, C. Wellbrock, R. Marais, Melanoma biology and new targeted therapy, Nature 445 (2007) 851.
[2] G.C. Leonardi, L. Falzone, R. Salemi, A. Zanghì, D.A. Spandidos, J.A. Mccubrey, S. Candido, M. Libra, Cutaneous melanoma: from pathogenesis to therapy, Int. J. Oncol. 52 (2018) 1071–1080.
[3] B.M. Tod, P.E. Kellett, E. Singh, W.I. Visser, C.J. Lombard, C.Y. Wright, The incidence of melanoma in South Africa: an exploratory analysis of National Cancer registry data from 2005 to 2013 with a specific focus on melanoma in black Africans, S. Afr. Med. J. 109 (2019) 246–253.
[4] C.M. Olsen, A.C. Green, N. Pandeya, D.C. Whiteman, Trends in melanoma incidence rates in eight susceptible populations through 2015, J. Invest. Dermatol. 139 (2019) 1392–1395.
[5] D.E. Godar, M. Subramanian, S.J. Merrill, Cutaneous malignant melanoma incidences analyzed worldwide by sex, age, and skin type over personal Ultraviolet-B dose shows no role for sunburn but implies one for Vitamin D3, Dermatoendocrinol. 9 (2017) e1267077.
[6] A. Millet, A.R. Martin, C. Ronco, S. Rocchi, R. Benhida, Metastatic melanoma: insights into the evolution of the treatments and future challenges, Med. Res. Rev. 37 (2017) 98–148.
[7] A. Amaro, R. Gangemi, F. Piaggio, G. Angelini, G. Barisione, S. Ferrini, U. Pfeffer, The biology of uveal melanoma, Cancer Metastasis Rev. 36 (2017) 109–140.

[8] W.E. Damsky, L.E. Rosenbaum, M. Bosenberg, Decoding melanoma metastasis, Cancers 3 (2011) 126–163.
[9] A.C. Wickremesekera, H.D. Brasch, V.M. Lee, P.F. Davis, K. Woon, R. Johnson, S.T. Tan, T. Itinteang, Expression of cancer stem cell markers in metastatic melanoma to the brain, J. Clin. Neurosci. 60 (2019) 112–116.
[10] A. Turdo, V. Veschi, M. Gaggianesi, A. Chinnici, P. Bianca, M. Todaro, G. Stassi, Meeting the challenge of targeting cancer stem cells, Front. Cell Dev. Biol. 7 (2019) 16.
[11] D. Ribatti, B. Nico, C. Floris, D. Mangieri, F. Piras, M.G. Ennas, A. Vacca, P. Sirigu, Microvascular density, vascular endothelial growth factor immunoreactivity in tumor cells, vessel diameter and intussusceptive microvascular growth in primary melanoma, Oncol. Rep. 14 (2005) 81–84.
[12] M.J. Hendrix, E.A. Seftor, A.R. Hess, R.E. Seftor, Angiogenesis: vasculogenic mimicry and tumour-cell plasticity: lessons from melanoma, Nat. Rev. Cancer 3 (2003) 411.
[13] B.A. Warren, P. Shubik, The growth of the blood supply to melanoma transplants in the hamster cheek pouch, Transplant. 4 (1966) 652.
[14] W.R. Hubler Jr., J.E. Wolf Jr, Melanoma. Tumor angiogenesis and human neoplasia, Cancer 38 (1976) 187–192.
[15] H. Erhard, F.J. Rietveld, E.B. Bröcker, D.J. Ruiter, Transition of horizontal to vertical growth phase melanoma is accompanied by induction of vascular endothelial growth factor expression and angiogenesis, Melanoma Res. 7 (1997) S19–26.
[16] J. Marcoval, A. Moreno, J. Graells, A. Vidal, J.M. Escribà, M. Garcia-Ramírez, A. Fabra, Angiogenesis and malignant melanoma. Angiogenesis is related to the development of vertical (tumorigenic) growth phase, J. Cutan. Pathol. 24 (1997) 212–218.
[17] A. Srivastava, P. Laidler, L.E. Hughes, J. Woodcock, E.J. Shedden, Neovascularization in human cutaneous melanoma: a quantitative morphological and Doppler ultrasound study, Eur. J. Cancer Clin. Oncol. 22 (1986) 1205–1209.
[18] W. Weninger, A. Uthman, J. Pammer, A. Pichler, C. Ballaun, I.M. Lang, A. Plettenberg, H.C. Bankl, M. Stürzl, E. Tschachler, Vascular endothelial growth factor production in normal epidermis and in benign and malignant epithelial skin tumors, Lab. Investig. J. Tech. Methods Pathol. 75 (1996) 647–657.
[19] S. Ilmonen, S. Asko-Seljavaara, A.L. Kariniemi, L. Jeskanen, S. Pyrhönen, T. Muhonen, Prognosis of primary melanoma, Scand. J. Surg. 91 (2002) 166–171.
[20] K.P. Claffey, L.F. Brown, L.F. del Aguila, K. Tognazzi, K.T. Yeo, E.J. Manseau, H.F. Dvorak, Expression of vascular permeability factor/vascular endothelial growth factor by melanoma cells increases tumor growth, angiogenesis, and experimental metastasis, Cancer Res. 56 (1996) 172–181.
[21] P.M. Lacal, C.M. Failla, E. Pagani, T. Odorisio, C. Schietroma, S. Falcinelli, G. Zambruno, S. D'Atri, Human melanoma cells secrete and respond to placenta growth factor and vascular endothelial growth factor, J. Invest. Dermatol. 115 (2000) 1000–1007.
[22] E.K. Rofstad, E.F. Halsør, Vascular endothelial growth factor, interleukin 8, platelet-derived endothelial cell growth factor, and basic fibroblast growth factor promote angiogenesis and metastasis in human melanoma xenografts, Cancer Res. 60 (2000) 4932–4938.
[23] V. Barak, J. Pe'er, I. Kalickman, S. Frenkel, VEGF as a biomarker for metastatic uveal melanoma in humans, Curr. Eye Res. 36 (2011) 386–390.
[24] J.R. Westphal, R. Van't Hullenaar, R. Peek, R.W. Willems, K. Crickard, U. Crickard, J. Askaa, I. Clemmensen, D.J. Ruiter, R.M. De Waal, Angiogenic balance in human melanoma: expression of VEGF, bFGF, IL-8, PDGF and angiostatin in relation to vascular density of xenografts *in vivo*, Int. J. Cancer 86 (2000) 768–776.
[25] Y.K. Chae, K. Ranganath, P.S. Hammerman, C. Vaklavas, N. Mohindra, A. Kalyan, M. Matsangou, R. Costa, B. Carneiro, V.M. Villaflor, M. Cristofanilli, Inhibition of the fibroblast growth factor receptor (FGFR) pathway: the current landscape and barriers to clinical application, Oncotarget 8 (2017) 16052.
[26] O. Straume, L.A. Akslen, Importance of vascular phenotype by basic fibroblast growth factor, and influence of the angiogenic factors basic fibroblast growth factor/fibroblast growth factor receptor-1 and ephrin-A1/EphA2 on melanoma progression, Am. J. Pathol. 160 (2002) 1009–1019.
[27] S. Ugurel, G. Rappl, W. Tilgen, U. Reinhold, Increased serum concentration of angiogenic factors in malignant melanoma patients correlates with tumor progression and survival, J. Clin. Oncol. 19 (2001) 577–583.
[28] M. Streit, M. Detmar, Angiogenesis, lymphangiogenesis, and melanoma metastasis, Oncogene 22 (2003) 3172.
[29] J.M. Conroy, S. Pabla, M.S. Ernstoff, I. Puzanov, M. Nesline, S.T. Glenn, A. Papanicolau-Sengos, B. Burgher, J. Andreas, V. Giamo, M. Qin, Comprehensive immune and mutational profile of melanoma, J. Clin. Oncol. 36 (2018) 182.
[30] M. Carleton, M. Zhou, O. De Henau, P. Phillips, T. Chen, Y. Feng, S.P. Huang, A. Walsh, T.P. Reilly, I.M. Bermejo, Serum interleukin 8 (IL-8) may serve as a biomarker of response to immuno-oncology (IO) therapy, J. Clin. Oncol. 36 (2018) 3025.
[31] A.J. Maniotis, R. Folberg, A. Hess, E.A. Seftor, L.M. Gardner, J. Pe'er, J.M. Trent, P.S. Meltzer, M.J. Hendrix, Vascular channel formation by human melanoma cells in vivo and in vitro: vasculogenic mimicry, Am. J. Pathol. 155 (1999) 739–752.
[32] E.A. Seftor, P.S. Meltzer, D.A. Kirschmann, J. Pe'er, A.J. Maniotis, J.M. Trent, R. Folberg, M.J. Hendrix, Molecular determinants of human uveal melanoma invasion and metastasis, Clin. Exp. Metastasis 19 (2002) 233–246.
[33] S. Frenkel, I. Barzel, J. Levy, A.Y. Lin, D.U. Bartsch, D. Majumdar, R. Folberg, J. Pe'er, Demonstrating circulation in vasculogenic mimicry patterns of uveal melanoma by confocal indocyanine green angiography, Eye 22 (2008) 948.
[34] R. Folberg, A.J. Maniotis, Vasculogenic mimicry, APMIS 112 (2004) 508–525.
[35] A.R. Hess, L.M. Postovit, N.V. Margaryan, E.A. Seftor, G.B. Schneider, R.E. Seftor, B.J. Nickoloff, M.J. Hendrix, Focal adhesion kinase promotes the aggressive melanoma phenotype, Cancer Res. 65 (2005) 9851–9860.
[36] J. Walker-Daniels, A.R. Hess, M.J. Hendrix, M.S. Kinch, Differential regulation of EphA2 in normal and malignant cells, Am. J. Pathol. 162 (2003) 1037.
[37] C.I. Schnegg, M.H. Yang, S.K. Ghosh, M.Y. Hsu, Induction of vasculogenic mimicry overrides VEGF-A silencing and enriches stem-like cancer cells in melanoma, Cancer Res. 75 (2015) 1682–1690.
[38] K.M. Hardy, D.A. Kirschmann, E.A. Seftor, N.V. Margaryan, L.M. Postovit, L. Strizzi, M.J. Hendrix, Regulation of the embryonic morphogen Nodal by Notch4 facilitates manifestation of the aggressive melanoma phenotype, Cancer Res. 70 (2010) 10340–10350.
[39] A. Vartanian, G. Gatsina, I. Grigorieva, E. Solomko, V. Dombrovsky, A. Baryshnikov, E. Stepanova, The involvement of Notch signaling in melanoma vasculogenic mimicry, Clin. Exp. Med. 13 (2013) 201–209.
[40] D.A. Kirschmann, E.A. Seftor, K.M. Hardy, R.E. Seftor, M.J. Hendrix, Molecular pathways: vasculogenic mimicry in tumor cells: diagnostic and therapeutic implications, Clin. Cancer Res. 18 (2012) 2726–2732.
[41] L.M. Postovit, E.A. Seftor, R.E. Seftor, M.J. Hendrix, Targeting Nodal in malignant melanoma cells, Expert Opin. Ther. Tar. 11 (2007) 497–505.
[42] M.J. Hendrix, I. Kandela, A.P. Mazar, E.A. Seftor, R.E. Seftor, N.V. Margaryan, L. Strizzi, G.F. Murphy, G.V. Long, R.A. Scolyer, Targeting melanoma with frontline therapy does not abrogate Nodal-expressing tumor cells, Lab. Investig. 97 (2017) 176–186.
[43] K.M. Hardy, L. Strizzi, N.V. Margaryan, K. Gupta, G.F. Murphy, Scolyer R.A, M.J. Hendrix, Targeting nodal in conjunction with dacarbazine induces synergistic anticancer effects in metastatic melanoma, Mol. Cancer Res. 13 (2015) 670–680.
[44] Z.-J. Liu, M. Xiao, K. Balint, K.S. Smalley, P. Brafford, R. Qiu, C.C. Pinnix, X. Li, M. Herlyn, Notch1 signaling promotes primary melanoma progression by activating mitogen-activated protein kinase/phosphatidylinositol 3-kinase-Akt pathways and up-regulating N-cadherin expression, Cancer Res. 66 (2006) 4182–4190.
[45] A. Gutierrez, A.T. Look, Notch and PI3K-AKT pathways intertwined, Cancer Cell 12 (2007) 411–413.
[46] Y. Funahashi, C.J. Shawber, A. Sharma, E. Kanamaru, Y.K. Choi, J. Kitajewski, Notch modulates VEGF action in endothelial cells by inducing Matrix Metalloprotease activity, Vasc. Cell 3 (2011) 2.
[47] A.R. Hess, E.A. Seftor, R.E. Seftor, M.J. Hendrix, Phosphoinositide 3-kinase regulates membrane Type 1-matrix metalloproteinase (MMP) and MMP-2 activity during melanoma cell vasculogenic mimicry, Cancer Res. 63 (2003) 4757–4762.
[48] R.E. Seftor, E.A. Seftor, N. Koshikawa, P.S. Meltzer, L.M. Gardner, M. Bilban, W.G. Stetler-Stevenson, V. Quaranta, M.J. Hendrix, Cooperative interactions of laminin 5 γ2 chain, matrix metalloproteinase-2, and membrane type-1-matrix/metalloproteinase are required for mimicry of embryonic vasculogenesis by aggressive melanoma, Cancer Res. 61 (2001) 6322–6327.
[49] L. Qiao, N. Liang, J. Zhang, J. Xie, F. Liu, D. Xu, et al., Advanced research on vasculogenic mimicry in cancer, J. Cell. Mol. Med. 19 (2015) 315–326.
[50] A.R. Hess, E.A. Seftor, L.M. Gruman, M.S. Kinch, R.E. Seftor, M.J. Hendrix, VE-cadherin regulates EphA2 in aggressive melanoma cells through a novel signaling pathway: implications for vasculogenic mimicry, Cancer Biol. Ther. 5 (2006) 228–233.
[51] D. Mitra, S. Bhattacharyya, N. Alam, S. Sen, Sa. Mitra, S. Mandal, S. Vignesh, B. Majumder, Nabendu Murmu, Phosphorylation of EphA2 receptor and vasculogenic mimicry is an indicator of poor prognosis in invasive carcinoma of the breast, Breast Cancer Res. Treat. 179 (2020) 359–370.
[52] Z.N. Demou, M.J. Hendrix, Microgenomics profile the endogenous angiogenic phenotype in subpopulations of aggressive melanoma, J. Cell. Biochem. 105 (2008) 562–573.
[53] B. Döme, M.J. Hendrix, S. Paku, J. Tóvári, J. Tímár, Alternative vascularization mechanisms in cancer: pathology and therapeutic implications, Am. J. Pathol. 170 (2007) 1–15.
[54] J.P. Yang, Y.D. Liao, D.M. Mai, P. Xie, Y.Y. Qiang, L.S. Zheng, M.Y. Wang, Y. Mei, D.F. Meng, L. Xu, L. Cao, Tumor vasculogenic mimicry predicts poor prognosis in cancer patients: a meta-analysis, Angiogenesis 19 (2016) 191–200.
[55] K. Spiliopoulos, D. Peschos, A. Batistatou, I. Ntountas, A. Papoudou-Bai, A. Zioga, N. Agnantis, G. Kitsos, Immunohistochemical study of vasculogenic mimicry and angiogenesis in melanocytic tumors of the eye and the periocular area, Anticancer Res. 37 (2017) 1113–1120.
[56] B. Sun, S. Zhang, D. Zhang, J. Du, H. Guo, X. Zhao, W. Zhang, X. Hao, Vasculogenic mimicry is associated with high tumor grade, invasion and metastasis, and short survival in patients with hepatocellular carcinoma, Oncol. Rep. 16 (2006) 693–698.
[57] M.A. Warso, A.J. Maniotis, X. Chen, D. Majumdar, M.K. Patel, A. Shilkaitis, T.K.D. Gupta, Prognostic significance of periodic acid-schiff-positive patterns in primary cutaneous melanoma, Clin. Cancer Res. 7 (2001) 473–477.
[58] A. Thies, U. Mangold, I. Moll, U. Schumacher, PAS-positive loops and networks as a prognostic indicator in cutaneous malignant melanoma, J. Pathol. 195 (2001) 537–542.
[59] Z. Cao, M. Bao, L. Miele, F.H. Sarkar, Z. Wang, Q. Zhou, Tumour vasculogenic mimicry is associatedwith poor prognosis of human cancer patients: a systemic review and meta-analysis, Eur. J. Cancer 49 (2013) 3914–3923.
[60] C.I. Baeten, F. Hillen, P. Pauwels, A.P. de Bruine, C.G. Baeten, Prognostic role of vasculogenic mimicry in colorectal cancer, Dis. Colon Rectum 52 (2009) 2028–2035.
[61] S. Williamson, F. Trapani, B. Abbott, M. Galvin, R. Metcalf, M. Hendrix, F. Blackhall, K. Frese, K. Simpson, C. Dive, The role of vasculogenic mimicry in small cell lung cancer, Eur. J. Cancer 61 (2016) S56.
[62] C. Lezcano, C.W. Lee, A.R. Larson, A.M. Menzies, R.F. Kefford, J.F. Thompson, M.C. Mihm Jr., S. Ogino, G.V. Long, R.A. Scolyer, G.F. Murphy, Evaluation of

stromal HGF immunoreactivity as a biomarker for melanoma response to RAF inhibitors, Mod. Pathol. 27 (2014) 1193.
[63] Y. Xu, Q. Li, X.Y. Li, Q.Y. Yang, W.W. Xu, G.L. Liu, Short-term anti-vascular endothelial growth factor treatment elicits vasculogenic mimicry formation of tumors to accelerate metastasis, J. Exp. Clin. Cancer Res. 31 (2012) 16.
[64] R. Folberg, V. Rummelt, R. Parys-Van Ginderdeuren, T. Hwang, R.F. Woolson, J. Pe'er, L.M. Gruman, The prognostic value of tumor blood vessel morphology in primary uveal melanoma, Ophthalmology 100 (1993) 1389–1398.
[65] M.J. Hendrix, E.A. Seftor, A.R. Hess, R.E. Seftor, Molecular plasticity of human melanoma cells, Oncogene 22 (2003) 3070.
[66] H. Han, L. Du, Z. Cao, B. Zhang, Q. Zhou, Triptonide potently suppresses pancreatic cancer cell-mediated vasculogenic mimicry by inhibiting expression of VE-cadherin and chemokine ligand 2 genes, Eur. J. Pharmacol. 818 (2018) 593–603.
[67] H. Ge, H. Luo, Overview of advances in vasculogenic mimicry–a potential target for tumor therapy, Cancer Manag. Res. 10 (2018) 2429.
[68] J. Meng, B. Sun, X. Zhao, D. Zhang, X. Zhao, Q. Gu, X. Dong, N. Zhao, P. Liu, Y. Liu, Doxycycline as an inhibitor of the epithelial-to-mesenchymal transition and vasculogenic mimicry in hepatocellular carcinoma, Mol. Cancer Ther. 13 (2014) 3107–3122.
[69] O. Itzhaki, E. Greenberg, B. Shalmon, A. Kubi, A.J. Treves, R. Shapira-Frommer, C. Avivi, R. Ortenberg, E. Ben-Ami, J. Schachter, M.J. Besser, Nicotinamide inhibits vasculogenic mimicry, an alternative vascularization pathway observed in highly aggressive melanoma, PLoS One 8 (2013) e57160.
[70] D. Milanović, F. Braun, W. Weber, A.L. Grosu, M. Behe, G. Niedermann, The influence of the combined treatment with Vadimezan (ASA404) and taxol on the growth of U251 glioblastoma xenografts, BMC Cancer 12 (2012) 242.
[71] J.Q. Guo, Q.H. Zheng, H. Chen, L. Chen, J.B. Xu, M.Y. Chen, D. Lu, Z.H. Wang, H.F. Tong, S. Lin, Ginsenoside Rg3 inhibition of vasculogenic mimicry in pancreatic cancer through downregulation of VE-cadherin/EphA2/MMP9/MMP2 expression, Int. J. Oncol. 45 (2014) 1065–1072.
[72] L. Asnaghi, D.T. White, L. Yoon, A. Price, G.Y. Lee, A. Sahoo, J.S. Mumm, C.G. Eberhart, Downregulation of Nodal inhibits metastatic progression in retinoblastoma, Acta Neuropathol. Commun. 7 (2019) 1–9.
[73] M. Su, Y.J. Feng, L.Q. Yao, M.J. Cheng, C.J. Xu, Y. Huang, Y.Q. Zhao, H. Jiang, Plasticity of ovarian cancer cell SKOV3ip and vasculogenic mimicry *in vivo*, Int. J. Gynecol. Cancer 18 (2008) 476–486.
[74] V. Makker, F.O. Recio, L. Ma, U.A. Matulonis, J.O. Lauchle, H. Parmar, H.N. Gilbert, J.A. Ware, R. Zhu, S. Lu, L.Y. Huw, Y. Wang, H. Koeppen, J.M. Spoerke, M.R. Lackner, C.A. Aghajanian, A multicenter, single-arm, open-label, phase 2 study of apitolisib (GDC-0980) for the treatment of recurrent or persistent endometrial carcinoma (MAGGIE study), Cancer 122 (2016) 3519–3528.
[75] R.L. Coleman, K.F. Handley, R. Burger, G.Z. Dal Molin, R. Stagg, A.K. Sood, K.N. Moore, Demcizumab combined with paclitaxel for platinum-resistant ovarian, primary peritoneal, and fallopian tube cancer: the SIERRA open-label phase Ib trial, Gynecol. Oncol. (2020) In press.
[76] A.W. Tolcher, W.C. Su, N. Lakhani, J. Gutheil, T.J. Melink, Y.W. Chu, Y.L. Chen, D.S. Chien, Phase I dose escalation study of CVM-1118, a novel anti-vascular mimicry agent, Patients with Advanced Cancers, (2017) 2580-2580.
[77] M. Surati, K. Valla, K.S. Shah, E.H. Panjic, S. Lonial, Panobinostat for the treatment of multiple myeloma, Expert Opin. Orphan D. (2015) 229–238.
[78] D. Wang, X. Dun, L. Yang, Y. Zhu, Y. Wang, Li, Current advance of therapeutic agents in clinical trials potentially targeting tumor plasticity, 9 Front. Oncol (2019) 887.
[79] A. Markham, Copanlisib: first global approval, Drugs 77 (2017) 2057–2062.
[80] J. Rodon, I. Braña, L.L. Siu, M.J. De Jonge, N. Homji, D. Mills, E. Di Tomaso, C. Sarr, L. Trandafir, C. Massacesi, F. Eskens, Phase I dose-escalation and-expansion study of buparlisib (BKM120), an oral pan-Class I PI3K inhibitor, in patients with advanced solid tumors, Invest. New Drug. 32 (2014) 670–681.
[81] J.P. Yang, Y.D. Liao, D.M. Mai, P. Xie, Y.Y. Qiang, L.S. Zheng, M.Y. Wang, Y. Mei, D.F. Meng, L. Xu, L. Cao, Tumor vasculogenic mimicry predicts poor prognosis in cancer patients: a meta-analysis, Angiogenesis. 19 (2016) 191–200.
[82] M. Yamakawa, S.J. Doh, S.M. Santosa, M. Montana, E.C. Qin, H. Kong, K.Y. Han, C. Yu, M.I. Rosenblatt, A. Kazlauskas, J.H. Chang, Potential lymphangiogenesis therapies: learning from current antiangiogenesis therapies—a review, Med. Res. Rev. 38 (2018) 1769–1798.
[83] S.A. Stacker, S.P. Williams, T. Karnezis, R. Shayan, S.B. Fox, M.G. Achen, Lymphangiogenesis and lymphatic vessel remodelling in cancer, Nat. Rev. Cancer 14 (2014) 159.
[84] B. Liu, J. Ma, X. Wang, F. Su, X. Li, S. Yang, W. Ma, Y. Zhang, Lymphangiogenesis and its relationship with lymphatic metastasis and prognosis in malignant melanoma, Anat. Rec. Adv. Integr. Anat. Evol. Biol. 291 (2008) 1227–1235.
[85] Z. Špirić, M. Vještica, M. Erić, Survival prediction in patients with cutaneous melanoma by tumour lymphangiogenesis, Acta Clin. Belg. (2019) 1–9.
[86] J. Rui-Cheng, Hypoxia and lymphangiogenesis in tumor microenvironment and metastasis, Cancer Lett. 346 (2014) 6–16.
[87] T. Han, J. Yan, H. Chen, Y. Ji, J. Chen, J. Cui, W. Shen, J. Zou, HIF-1α contributes to tube malformation of human lymphatic endothelial cells by upregulating VEGFR-3, Int. J. Oncol. 54 (2019) 139–151.
[88] E. Ayubi, S. Safiri, Lymphatic vessel density and VEGF-C expression as independent predictors of melanoma metastases: methodological issues, J. Plast. Reconstr. Aesthet. Surg. 71 (2018) 604–605.
[89] J.S. Goydos, D.H. Gorski, Vascular endothelial growth factor C mRNA expression correlates with stage of progression in patients with melanoma, Clin. Cancer Res. 9 (2003) 5962–5967.
[90] M.G. Achen, M. Jeltsch, E. Kukk, T. Mäkinen, A. Vitali, A.F. Wilks, K. Alitalo, S.A. Stacker, Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4), Proc. Natl. Acad. Sci. 95 (1998) 548–553.
[91] Z. Špirić, Ž. Eri, M. Erić, Significance of vascular endothelial growth factor (VEGF)-C and VEGF-D in the progression of cutaneous melanoma, Int. J. Surg. Pathol. 23 (2015) 629–637.
[92] S.D. Nathanson, K. Rosso, D. Chitale, M. Burke, Lymph node metastasis, Introduction to Cancer Metastasis, Elsevier, 2017, pp. 235–261.
[93] M.S. Emmett, S. Lanati, D.B. Dunn, O.A. Stone, D.O. Bates, CCR7 mediates directed growth of melanomas towards lymphatics, Microcirculation 18 (2011) 172–182.
[94] L. Kühnelt-Leddihn, H. Müller, K. Eisendle, B. Zelger, G. Weinlich, Overexpression of the chemokine receptors CXCR4, CCR7, CCR9, and CCR10 in human primary cutaneous melanoma: a potential prognostic value for CCR7 and CCR10? Arch. Dermatol. Res. 304 (2012) 185–193.
[95] B.J. Chi, C.L. Du, Y.-F. Fu, Y.-N. Zhang, R.W. Wang, Silencing of CCR7 inhibits the growth, invasion and migration of prostate cancer cells induced by VEGFC, Int. J. Clin. Exp. Pathol. 8 (2015) 12533.
[96] Y. He, Angiopoietins and tie receptors in lymphangiogenesis and tumor metastasis, Tumor Angiogenesis: A Key Target for Cancer Therapy, Springer, 2019, pp. 135–156.
[97] L.C. Dieterich, M. Detmar, Tumor lymphangiogenesis and new drug development, Adv. Drug Deliv. Rev. 99 (2016) 148–160.
[98] G.S. Falchook, J.W. Goldman, J. Desai, I. Leitch, D.S. Hong, V. Subbiah, R. Kurzrock, L.S. Rosen, A first-in-human phase I study of VGX-100, a selective anti-VEGF-C antibody, alone and in combination with bevacizumab in patients with advanced solid tumors, J. Clin. Oncol. (2014) 2524-2524.
[99] M.W. Saif, J.A. Knost, E.G. Chiorean, S.R.P. Kambhampati, D. Yu, B. Pytowski, A. Qin, J.S. Kauh, B.H. O'Neil, Phase 1 study of the anti-vascular endothelial growth factor receptor 3 monoclonal antibody LY3022856/IMC-3C5 in patients with advanced and refractory solid tumors and advanced colorectal cancer, Cancer Chemother. Pharmacol. 78 (2016) 815–824.
[100] D.A. Reardon, A.B. Lassman, D. Schiff, S.A. Yunus, E.R. Gerstner, T.F. Cloughesy, E.Q. Lee, S.C. Gaffey, J. Barrs, J. Bruno, A. Muzikansky, Phase 2 and biomarker study of trebananib, an angiopoietin-blocking peptibody, with and without bevacizumab for patients with recurrent glioblastoma, Cancer 124 (2018) 1438–1448.
[101] N. Molnar, D.W. Siemann, Angiopoietin-2 axis inhibitors: current status and future considerations for cancer therapy, Curr. Angiogenes. 2 (2013) 2–12.
[102] S. Rivera, C. Leteur, F. Mégnin, et al., Time dependent modulation of tumor radiosensitivity by a pan HDAC inhibitor: abexinostat, Oncotarget 8 (34) (2017) 56210–56227.
[103] R. Aggarwal, S. Thomas, N. Pawlowska, et al., Inhibiting histone deacetylase as a means to reverse resistance to angiogenesis inhibitors: phase I study of Abexinostat plus Pazopanib in advanced solid tumor malignancies, J. Clin. Oncol. 35 (11) (2017) 1231–1239.