Published in final edited form as:
*Semin Oncol*. 2007 December ; 34(6): 555–565.

# Angiogenesis in Melanoma

**Ganapati H. Mahabeleshwar**[1] and **Tatiana V. Byzova**[1¶]

1*Department of Molecular Cardiology, J.J. Jacobs Center for Thrombosis and Vascular Biology, NB50, The Cleveland Clinic Foundation, 9500 Euclid Ave, Cleveland, OH 44195.*

## Summary

The process of angiogenesis is crucial for progression and metastasis of the majority of solid tumors including melanomas. The purpose of this review is to summarize existing knowledge of the mechanisms of angiogenesis in melanoma as well as current anti-angiogenic therapeutic strategies and their targets. Here, we have focused primarily on the role of key growth factors which are secreted by melanoma cells and known to trigger angiogenic responses, and their receptors as expressed on both endothelial and melanoma cells. Many of these growth factors function in synergy with receptors for extracellular matrix, integrins and matrix metalloproteinases. All these systems of molecules are activated during major stages of angiogenesis such as endothelial migration, proliferation and reorganization of surrounding extracellular matrix. The blockade of these molecules and their downstream pathways leads to inhibition of melanoma vascularization. Thus, these classes of molecules are essential for melanoma angiogenesis and, therefore, might serve as promising targets for therapeutic intervention. Many recently developed compounds targeting key pathways in angiogenesis are in their final stages of clinical trials.

### Keywords

Melanoma; Angiogenesis; Extracellular matrix; Growth factors; Integrins; Matrix metalloproteinase

## Introduction

Angiogenesis, the process of formation of neovasculature from pre-existing blood vessels, is widely considered as an essential process to ensure the supply of nutrients and oxygen to rapidly growing tumors as well as to provide a route for tumor cell metastasis[1]. Angiogenesis is a distinct feature of several human cutaneous melanomas and was first described by Warren and Shubik following transplantation of human melanoma tumor tissue into a hamster cheek pouch[2]. These studies were later confirmed by Hubler and Wolf [3]. Rapid angiogenesis of cutaneous melanomas dramatically enhances the risk of lethality and contributes to the progression of the most common type of cancer in young adults.

Like any other cutaneous neoplasia, melanomas follow discrete sequential transformation processes in which the nevus attains dysplastic radial growth phase (Figure.1). This is followed by a vertical growth phase[4–6], The vertical growth phase requires high angiogenic activity, which, in turn, contributes to melanoma cell metastasis. However, the most critical step in

**¶Corresponding author:** Tatiana V. Byzova, Joseph J. Jacobs Center for Thrombosis and Vascular Biology, Department of Molecular Cardiology, Cleveland Clinic Foundation, NB50, 9500 Euclid Avenue, Cleveland, OH 44195. Telephone: 216-445-4312; Fax: 216-445-8204; e-mail: byzovat@ccf.org.

metastasis is the spreading of melanomas to lymphatic vessels surrounding the tumor tissue. Following lymph node invasion, melanoma cells metastasize to the lung, liver, cerebrum, and other sites. Exposure to ultraviolet radiation is known to cause genetic changes in skin, which modulate the cutaneous immune response and increase production of several growth factors [7–9]. This causes uncontrolled proliferation of melanocytes, which, in turn, dramatically increases the consumption of oxygen and nutrients eventually leading to cell starvation and hypoxia[10,11]. To fulfill this increasing demand additional vasculature needs to be developed. Thus, to increase the blood supply, the tissue begins to produce a spectrum of growth factors which trigger the process of angiogenesis.

Studies using human melanoma xenograft models in nude mice indicated that melanoma tumor cells serve as a source of several growth factors including but not limited to vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), two powerful stimulators of rapid angiogenesis[12]. Despite recent advances in our understanding of the molecular mechanisms of tumor growth/metastasis and the availability of modern technology to detect and treat various human cancers, our knowledge about the mechanisms underlying cutaneous tumor angiogenesis and metastasis remains rudimentary.

In this review we have focused on the role of cytokines and growth factors secreted by melanoma cells in tumor angiogenesis. These growth factors are involved in both autocrine and paracrine regulation of melanoma progression. Importantly, growth factors and their receptors function in intimate relationship with extracellular matrix proteins, their receptors, integrins, and matrix metalloproteinases. These classes of molecules are essential for melanoma angiogenesis and, therefore, serve as promising targets for therapeutic intervention. Most of the relatively recently developed compounds are at the final stages of clinical trials. Thus, this review is intended to summarize the basic biological knowledge regarding melanoma tumor angiogenesis as well as current clinical approaches to treat late stages of malignant melanomas.

## Growth factor dependent melanoma tumor growth and angiogenesis

Angiogenesis in melanoma is stimulated by a variety of growth factors such as VEGF, basic FGF, acidic FGF, platelet derived growth factor (PDGF), and transforming growth factors α and β (TGF-α and β) are among the others (Figure 2). Angiogenesis serves as a turning point in melanoma tumor growth and metastasis.

### VEGF and FGF

Transformed melanocytes are known to produce high amounts of bFGF as well as VEGF during conversion of the dysplastic naevus to the vertical growth phase. This extensive growth factor production continues until new blood vessel formation is complete. Recent reports indicate that basal expression of VEGF by transformed melanocytes plays a more important role in promoting angiogenesis in melanomas than hypoxia-induced VEGF up-regulation[13–15]. At the same time, bFGF regulates endothelial cell proliferation and angiogenesis by both autocrine and paracrine mechanisms. Since bFGF is devoid of the classic signal peptide for secretion, tumor cells release this factor by exocytosis from endoplasmic reticulum. Significant amounts of bFGF were found to be associated with extracellular matrix as well as with basement membrane of the newly formed blood vessels in human melanomas[16]. Digestion of extracellular matrix by matrix metalloproteinases of melanoma or endothelial origin promotes release of matrix bound bFGF, which, in turn, stimulates endothelial cell proliferation and vascular tube formation in melanomas[17,18]. Therefore drugs targeting these molecules are currently under various stages of clinical trial (Table 1).

## PIGF

Another important stimulator of melanoma angiogenesis is placental growth factor (PIGF). PIGF exists in several isoforms that are generated by alternative splicing of the same gene product and it exhibits distinct heparin-binding properties. PIGF-1 and -2 are expressed by melanoma cells and are known to bind to neuropilin-1 and -2 receptors expressed on endothelial cells[19]. In addition, PIGF acts through binding to VEGF receptor-1. Also, PIGF forms heterodimers with VEGF and, as a result, is able to bind to VEGF receptor-2 on endothelial cells (Figure 2). PIGF enhances melanoma tumor angiogenesis not only by acting on pre-existing endothelial cells, but also by mobilization and recruitment of VEGFR-1 positive hematopoietic precursors from bone marrow[20]. Further, PIGF is known to contribute to angiogenesis by acting on VEGFR-1-expressing smooth muscle cells/pericytes, thereby enhancing blood vessel maturation. PIGF secreted by both primary and metastatic melanomas induces tumor cell proliferation, indicating PIGF might contribute to melanoma tumor growth through an autocrine mechanism. Several studies also indicated that PIGF mediated neovascularization resulted in rapid vessel branching and formation of large and stable blood vessels in various in vivo models[21].

## IL-8 and TGF-1

Several growth factors and their receptors are implicated in the pathogenesis of a variety of human melanomas since their upregulation is associated with melanoma progression. Among these is interleukin-8 (IL-8). Its expression was found to be very minimal in normal epidermis and benign melanocytic lesions. However, it is dramatically increased in a majority of cutaneous melanomas examined. In a clinical study analysis IL-8 serum levels in melanoma patients are significantly elevated compared to healthy individuals[22]. Further, serum IL-8 levels correlate with advanced disease stage as well as with overall survival of melanoma patients. A recent study by Liu et al[23] indicated that transforming growth factor-1 (TGF-1) is able to enhance expression of IL-8 in human melanoma cells and promote angiogenesis in several mouse xenograft models. Several studies conclusively demonstrated that while tumor cell-derived IL-8 directly supports the growth of melanomas, IL-8 of endothelial origin further promotes melanoma cell migration. Additionally, tumor-derived IL-8 is able to induce endothelial cell migration (Figure 2).

IL-8 is also known to modulate vascular permeability. It functions through activation of G-protein coupled receptors on endothelial cells, leading to enhanced actin stress fiber formation, which, in turn, results in cell retraction and gap formation between endothelial cells Experimental evidence from several laboratories indicated that over-expression of IL-8 in poorly vascularized and nonmetastatic melanoma cells resulted in enhanced angiogenesis, rapid tumor growth and increased metastatic potential of melanomas[24,25].

## Plasminogen system

Besides a number of growth factors discussed above, there are other important players in melanoma which might eventually serve as therapeutic targets. Urokinase plasminogen activator and its receptor (uPA/uPAR) have been demonstrated to play a crucial role in several stages of melanoma tumor progression including melanoma cell migration, invasion and metastasis[26]. At the same time, uPA secreted from melanoma tumor cells is able to regulate endothelial cell functions including migration and the organization of endothelial cells into tube-like structures. Analysis of biopsy specimens of skin lesions indicated that uPA expression highly correlated with disease progression in a majority of patients. Delbaldo et al[27] demonstrated that uPA and plasminogen activator inhibitor type 1 mRNAs accumulate in atypical naevocytes and in melanoma cells, but not in benign naevocytes. Further, these observations suggest that up-regulation of the uPA gene is an early event of melanocyte transformation and that unbalanced enzyme activity is associated with the malignant

phenotype. Studies from Hearing et al[28] indicated that mouse melanoma cells express the single chain form of uPA on their cell surface and that these cells are capable of plasminogen-dependent extracellular matrix degradation. Further, melanoma cells that had been treated with anti-uPA antibody showed significantly decreased metastatic activity compared to controls. Studies from Min et al[29] demonstrated that molecules which prevent uPA binding to its receptor are able to inhibit bFGF-induced neovascularization in vivo and mouse melanoma tumor growth in syngeneic mice. Additional studies also demonstrated inhibition of melanoma tumor cell metastasis by uPA inhibitors in similar models. Taken together, these studies suggest that uPA/uPAR system is required for melanoma tumor angiogenesis and growth, as well as metastasis (Figure 2). Therefore, antagonists of the uPA/uPAR system might be utilized as potential inhibitors of tumor progression through several mechanisms[30,31].

## Integrin signaling in melanoma tumor progression and angiogenesis

Integrins are heterodimeric transmembrane glycoproteins consisting of α and β subunits. Their large ectodomain is required for ligand binding, while a short cytoplasmic domain transfers signals from the extracellular environment to the cytoplasm via a short transmembrane domain (Figure 3). Integrins mediate cellular processes such as migration, invasion, proliferation and metastasis in a variety of human cancers including melanomas[32]. Even though integrins are classically described as cell adhesion molecules, recent advances in integrin biology indicate that they play a very significant role in signal transduction, gene expression, cellular proliferation and regulation of apoptosis or anoikis of normal as well as transformed cells (Figure 3). Beyond this, integrins also play very crucial roles in embryogenesis, inflammation, immunity, tissue differentiation, regulation of cell growth, hemostasis and angiogenesis[33].

Integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ are known to upregulate on endothelial cells during tumor neovascularization[34]. Several $\alpha_v\beta_3$ integrin ligands including vitronectin, osteopontin and bone sialoprotein are known to modulate both VEGF as well as FGF-2-induced tumor angiogenesis in mouse models[35]. Studies from several groups indicate that $\alpha_v\beta_3$ integrin plays a very crucial role in the progression of cutaneous melanomas from the benign radial growth phase to the metastatic vertical growth phase[36]. Studies utilizing $\alpha_v\beta_3$ integrin inhibitory peptides, antibodies or small molecular compounds indicate that $\alpha_v\beta_3$ integrin indeed is required for tumor endothelial cell survival, therefore its inhibition leads to the regression of tumor vasculature[37]. These studies further indicate that the expression of $\alpha_v$, $\alpha_2$, $\alpha_3$ and $\alpha_4$ integrin subunits are elevated in malignant melanoma tumor cells compared to normal melanocytes. Similarly, laminin receptor $\alpha_6\beta_4$ integrin expression is significantly reduced in melanoma cells compared to benign cells[38]. In contrast, clinical samples of human malignant tissue show a significant increase in $\alpha_6\beta_4$ expression[39]. Therefore, the role of $\alpha_6\beta_4$ integrin in melanoma tumor growth and metastasis is controversial. Among the beta subunits, expression of $\beta_1$ and $\beta_3$ are more frequently elevated in several tumor types, including melanomas (Table 2).

Other major functions assigned to integrins expressed by melanoma cells are to support ECM anchorage-dependent tumor cell proliferation and anchorage-independent invasion. Integrins are also crucial in tumor angiogenesis and tumor cell metastasis[40]. Expression analysis at different stages of melanoma progression have indicated that the levels of $\beta_3$ and $\beta_1$ integrins promote melanoma transition from the radial growth phase to the vertical growth phase[41]. This phenomenon is further connected with rapid tumor neovascularization as well as tumor cell metastasis. Another interesting phenomenon observed in melanoma patients is the enhanced expression of integrin ligands. One such $\alpha_v\beta_3$ integrin ligand is osteopontin[42]. Constitutive expression of osteopontin in dermal cells leads to sustained anti-apoptotic signaling in melanocytes and a high rate of proliferation. A recent study utilizing numerous clinical samples of melanomas in various clinical grades indicated that the expression of osteopontin is

positively correlated with an invasive phenotype[43]. Further clinical studies have indicated that serum osteopontin levels are significantly higher in patients with benign or metastatic tumors compared to healthy individuals[44]. Therefore, serum osteopontin levels could potentially be a prognostic marker for various human cancers.

Studies from various laboratories indicate that expression levels of $\alpha_2\beta_1$ and $\alpha_5\beta_1$ are higher during the vertical growth phase of melanoma compared to the radial growth phase or malignant transformation[45–47]. Melanoma cells during the vertical growth phase bind to collagen type-1 through $\alpha_2\beta_1$ and $\alpha_5\beta_1$ integrins (Table 2). This stimulates the expression of MMP-1 and -2, which are crucial for collagen fibril degradation in the dermis which in turn facilitates vertical spreading of melanomas. The accumulated, denatured collagen acts as the $\alpha_v\beta_3$ integrin ligand as the denaturation process exposes RGD sequences[48]. The binding of denatured collagen to $\alpha_v\beta_3$ integrin further stimulates MMP-2 expression in these cells, increasing their invasive potential. One of the major photolytic degradation products due to over-expression of MMPs in dermis is fibronectin. The fibronectin receptor $\alpha_5\beta_1$ is expressed abundantly in most melanomas studied. Over- expression of $\alpha_5\beta_1$ in mouse melanoma cells leads to enhanced expression of MMP-2 and MMP-7[49]. To summarize, activation of integrins leads to enhanced expression of MMPs, which in turn degrade ECM components thereby generating a series of integrin ligands. These integrin ligands generated by photolytic degradation bind to the invasive melanomas and activate the signaling cascades required for malignant transformation.

As integrins are known to positively regulate angiogenesis, tumor growth and metastasis, several inhibitors of integrins are currently under clinical trials[50]. Most clinical trials are focused on inhibitors of the $\alpha_v\beta_3$ integrin complex or $\alpha_v$ integrin alone[37]. These include cilengitide, ATN-161, CNTO-95 and vitaxin (the last two are humanized monoclonal antibodies). These compounds specifically mask ligand binding sites and promote the internalization of targeted integrins. Peptide integrin inhibitors currently under clinical trials include cilengitide and ATN-161. Cilengitide is a cyclic RGD peptide that specifically inhibits $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin function. In preclinical studies, cilengitide substantially reduced tumor growth in a mouse melanoma xenograft model. Even though cilengitide has been in clinical trials for some time, the outcome of this trial has yet be published[34]. Similarly, the peptide integrin inhibitor ATN-161 is still under phase 1 clinical trials and data indicates that it exhibits anti-angiogenic and anti-metastatic activities[51]. Results of phase 1 clinical trials of vitaxin indicated that it stabilized disease and reduced the risk of metastases. However, results of phase 2 clinical trials indicated a very modest response and were not very encouraging. Therefore, in recent phase 2 clinical trials, vitaxin was administrated in combination with a standard chemotherapeutic compound (dacarbazine). Beyond this, several integrin inhibitors including small molecule compounds are currently in the preclinical phase of development. E7820, an aromatic sulfonamide derivative known to inhibit $\alpha_2$ integrin, entered its phase 1 clinical trial in early 2004[50]. Similarly, volociximab, a humanized monoclonal antibody specifically targeting $\alpha_5\beta_1$ integrin is also currently in phase 2 clinical trial. Phase 1 clinical studies aimed to determine the optimal concentration did not find any dose limiting toxicity of this antibody. Overall, integrin inhibitor clinical trials are encouraging and several small molecule compounds have successfully completed preclinical studies.

## Role of matrix metalloproteinases in melanoma angiogenesis

Matrix metalloproteinases (MMPs) are the major class of proteases that play crucial roles in tissue remodeling during embryonic development, bone resorption, wound healing and angiogenesis. Their catalytic activity is regulated partially by tissue inhibitors of matrix metalloproteinases and it has been demonstrated by several research groups that MMPs produced by actively proliferating tumor cells facilitate angiogenesis, tumor growth and metastasis[52]. As MMPs are actively involved in efficient matrix degradation, MMP expression

and catalytic activity are tightly regulated, at the stages of transcription, post-translational extracellular activation, and by suppression by its inhibitors[53]. Several studies indicated that the basal level of MMP production in benign or normal melanocytes is typically low and expression of MMPs is highly correlated with disease progression. MMP activation is accomplished by removal of the N-terminal propeptide domain through exogenous or autocatalytic cleavage[54]. Previous studies demonstrated that serine proteases such as plasmin activate most of the MMPs through this mechanism. MMP-2, which is abundantly expressed in early stages of malignant transformation, is known to achieve activation in a membrane-associated manner in endothelial cells and melanoma tumor cells. Further, cell surface associated membrane-type matrix metalloproteinase (MT1-MMP) is also known to activate MMP-2 through this mechanism[55]. To date the most extensively studied MMPs in melanomas are MMP-2 and MMP-9. It has been demonstrated by several groups that the expression and activation of these enzymes has been correlated to the invasive and metastatic phenotypes of melanomas[56]. Previous reports indicated that MMP-2 and MMP-9 are constitutively expressed in malignant melanomas and their expression is highly associated with melanoma atypia and dedifferentiation in melanocytic lesions[57].

Currently, cell surface associations of secreted MMPs through post translational modification have developed wide interest in the scientific community. It has been previously demonstrated that MMP-2/TIMP-2 associated with the cell surface in melanomas exhibits enhanced catalytic activity against its substrates compared to MMPs in secreted phase. Malignant melanoma cells are known to express a number of MMPs, including MMP-1, -2, -9, -13, and -14, as well as inhibitors of MMPs such as TIMP-1, -2 and -3[56]. A recently published study from Kerkela et al clearly demonstrates a specific distribution of MMPs within cutaneous squamous cell carcinomas[57]. Another recent clinical study also indicated that increased MMP-2 expression in melanomas was highly correlated with metastasis. Further, increases in expression of MMPs were shown to highly correlate with low survival rates in patients with malignant melanoma tumors[58]. It is also very important to note that not only expression of MMPs, but also their functional activity, is required for malignant tumor progression. Genetic overexpression of MT1-MMP in melanoma cells induced activation of MMP-2 and this activation is crucial for extracellular matrix degradation when localized on the leading edge of invasive carcinomas. In a clinical study of human melanoma lesions consisting of different stages of tumor progression it was found that MMP-2 and MT1-MMP positive tumor cells were often restricted to the interface between the tumor stroma and the invasive part of the tumor[57]. Surprisingly, expression of MMPs is not restricted to tumor cells but is also found abundantly in stromal cells. This clearly indicates a major contribution of host-derived proteases to melanoma tumor progression.

One of the major MMPs found to be expressed in human melanoma is MMP-1[59]. A series of studies has also indicated that MMP-1 expression is highly associated with malignant melanoma progression. In vitro studies indicated that degradation of collagen types I and IV and tumor cell invasion through Matrigel required MMP-1 expression (Table 3). Other than MMP-1 and -2, the major MMP expressed in melanoma tumor cells is MMP-9 which is also referred to as gelatinase B[60]. MMP-9 expression in melanoma tumor cells was found exclusively during the horizontal growth phase but not during the vertical growth phase. This clearly suggests that expression of MMP-9 is an early event in melanoma progression. Studies using a mouse model indicated that MMP-9 expression was only detected in advanced stages of disease, not in early melanocyte lesions[61]. Further, melanomas expressing constitutively high levels of MMP-9 exhibited increased lung colonization in experimental lung metastasis models. These advancements in understanding of MMP-9 biology indicate that MMPs expressed either by neoplastic or stromal cells are important in the metastasis of melanomas[62].

Several studies using either model cell lines or animals have demonstrated that the balance between MMPs and their inhibitors finally determines melanoma tumor progression[63–68]. To date, tissue inhibitors of matrix metalloproteinases (TIMPs) are extensively studied as they are natural inhibitors of MMPs and hence could be potential therapeutic targets. Numerous conclusive studies demonstrate that overexpression of (TIMP) -1,-2 and -3 significantly reduces melanoma tumor cell invasion, migration, tumor growth and metastasis[69]. Further, several studies have indicated that TIMPs significantly reduce tumor neovascularization in the several tumor models studied. Even though TIMPs are known to inhibit tumor cell metastasis in several experimental animal models, in human melanoma cells TIMP expression significantly enhances tumor cell proliferation[70]. Therefore, the role of TIMPs in melanoma tumor growth remains controversial.

As MMPs are known to play very crucial roles during the processes of tumor progression, several inhibitors specifically targeting MMPs are currently undergoing clinical trials. In the early '90s MMP inhibitors generated great enthusiasm among several research groups wishing to take them to clinical trials. Preclinical trials of MMP inhibitors were very promising, showing minimum side effects compared to other drugs available at the time[71]. Several current inhibitors, which have been tested in preclinical and clinical trials, are broad category MMP inhibitors. Pharmacological inhibitors such as batimastat and its analog marimastat, which interfere with the catalytic site of the MMPs, were the first inhibitors studied in detail. A recent review by Coussens et al discusses the status of several MMP inhibitors in clinical trials[72]. The first clinical trial of MMP inhibitors started in 1997 with marimastat and prinomastat. Phase 1 and 2 clinical trials were mostly focused on the optimal biological dose of MMP inhibitor rather than clinical outcome. Phase 2 and 3 clinical trials include three major strategies: (1) the clinical outcomes of MMP inhibitors were directly compared with standard chemotherapy. (2) MMP inhibitors were used in combination with standard chemotherapy. (3) Effects of MMP inhibitors were directly compared with placebo, which was administered in those patients who had no clinical evidence of disease after standard chemotherapy[71]. Therefore, the results of the MMP inhibitor clinical trials were very conclusive. Most of the MMP inhibitors tested in clinical trials were not very promising due to the lack of positive outcomes and the appearance of substantial drug side effects, which were not observed in preclinical studies. Therefore, most of the MMP inhibitor clinical trials were terminated following phase 3 clinical trials[71]. This failure of early MMP inhibitor clinical trials substantially suppressed the initiation of new clinical trials of MMP inhibitors targeting cancer.

## Anti-angiogenic therapies in melanoma

Tumor vasculature is essential for a tumor's growth, progression and metastasis to distant sites. According to the original theory of Judah Folkman, the destruction of tumor vasculature must initiate the process of tumor suppression and death[73]. Based on the results of the most recently published studies, inhibition of angiogenesis might make tumors highly susceptible to radiation and chemotherapy[74]. Most importantly, the process of angiogenesis generally is not required for the normal physiology of normal adult organisms, with the exception of the female reproductive cycle or recovery from an injury. Therefore, minimal side effects are anticipated from the inhibition of this process. Based on preclinical data, one might predict the side effects will be restricted to impaired wound healing, a process known to be dependent on angiogenesis. Taken together, the predicted specificity and effectiveness of the approach initiated an interest in angiogenesis as a therapeutic target.

It is not surprising that numerous anti-angiogenic agents are currently in clinical trials or in development[75–78]. Table 4 shows several classes of anti-angiogenic compounds divided into groups based on their molecular specificity. The list of molecular targets for anti-angiogenesis therapy includes: 1) Components or fragments of extracellular matrix and metalloproteinase

inhibitors[79,80]; 2) Angiogenic growth factors and their receptors (expressed both on tumor as well as on endothelial cells). This category includes monoclonal antibodies and soluble forms of receptors designed to bind and neutralize growth factors, small molecules designed to inhibit growth factor-receptor interaction or tyrosine kinase activity of receptors[81–83]. In addition, we included oligosaccharide-based inhibitors of growth factor release from extracellular matrix into this category. 3) A class of compounds which targets cellular receptors for extracellular matrix, integrins expressed on both melanoma and endothelial cells[84]. There are monoclonal antibodies, peptides and small molecule inhibitors in this category. All of the drugs presented in Table 4 have demonstrated significant inhibition of tumor angiogenesis and tumor growth in preclinical studies. Many experimental models included melanoma[85].

At present, it is quite intriguing to follow the results of clinical studies. Not all of the trials have been reported and discussed in detail in the literature. As a result, the overall picture of the efficacy of various groups of anti-angiogenic compounds is constantly changing, making any sort of generalization challenging. An interest in anti-angiogenic therapy dramatically increased after the US Food and Drug Administration (FDA) approved bevacizumab (Avastin) for several forms of cancers[86]. Inhibition of angiogenesis by bevacizumab appears to be a promising and effective strategy for solid tumors. It has been successfully used alone as well as in combination with chemotherapy treatments in several types of cancers[86]. Bevacizumab is relatively well tolerated; however, some observed side effects were surprising and not predicted based on the known mechanism of action for this antibody. Thus, it appears that additional preclinical studies will be required to fully address the effects of bevacizumab and its underlying mechanism on normal functional characteristics of vascular and hemostatic systems. A PUBMED search using the combination of key words "bevacizumab and melanoma" in the clinical trials category resulted in "0" hits. However, based on information from the NIH website "www.ClinicalTrials.gov" it appears that at least seven studies of bevacizumab as monotherapy or in combination with other compounds (carboplatin, paclitaxel, sorafenib, dacarbazine, interferon-alpha, imatinib were listed) in melanoma are currently recruiting patients. Results of bevacizumab clinical trials in melanoma are listed as "to be published" on http://www.centerwatch.com/patient/trialresults/druglst_Avastin_bevacizumab.html Since bevacizumab was the first FDA approved anti-angiogenic compound, it will most likely serve as a gold standard for other therapies which are currently in development. However, it is clear that drugs designed to target the same pathway, or even the same molecule, are not anticipated to display similar efficacies or similar profiles of side effects in melanoma or other cancer patients.

In sum, the process of angiogenesis in melanoma is crucial for tumor development and metastasis and undoubtedly requires further detailed investigation of its underlying mechanisms. Since this process involves a synergistic action of several classes of molecules and signaling pathways, there are numerous possibilities for the development of non-overlapping therapeutic strategies. Several angiogenic compounds are already in clinical trials for melanoma and available preliminary results are encouraging.

**Acknowledgements**

We acknowledge financial support from the US National Institutes of Health (HL071625 and HL073311 to TVB) and American Heart Association (0625271B to GHM).

## References

1. Folkman J. Angiogenesis. Annu Rev Med 2006;57:1–18. [PubMed: 16409133]
2. Warren BA, Shubik P. The growth of the blood supply to melanoma transplants in the hamster cheek pouch. Lab Invest 1966;15(2):464–478. [PubMed: 5932611]

3. Hubler WR Jr, Wolf JE Jr. Melanoma. Tumor angiogenesis and human neoplasia. Cancer 1976;38(1): 187–192. [PubMed: 947514]
4. Elder DE, Van Belle P, Elenitasas R, Halpern A, Guerry D. Neoplastic progression and prognosis in melanoma. Semin Cutan Med Surg 1996;15:336–348. [PubMed: 9069601]
5. Meier F, Satyamoorthy K, Nesbit M, Hsu MY, Schittek B, Garbe C, Herlyn M. Molecular events in melanoma development and progression. Front Biosci 1998;3:D1005–D1010. [PubMed: 9740553]
6. Tuthill RJ, Reed RI. Failure of Senescence in the Dysplasia-Melanoma Sequence: Demonstration using a Tissue Microarray and a Revised Paradigm for Melanoma. Semin Oncology. 2007
7. Jhappan C, Noonan FP, Merlino G. Ultraviolet radiation and cutaneous malignant melanoma. Oncogene 2003;22:3099–3112. [PubMed: 12789287]
8. Lin JY, Fisher DE. Melanocyte biology and skin pigmentation. Nature 2007;445:843–850. [PubMed: 17314970]
9. Cooper K, et al. Semin Oncology. 2007 Jul 22;
10. Michaylira CZ, Nakagawa H. Hypoxic miroenvirornment as a cradle for melanoma development and progression. Cancer Biol Ther 2006;5:476–479. [PubMed: 16627974]
11. Hendrix MJ, Seftor EA, Seftor RE, Kasemeier-Kulesa J, Kulesa PM, Postovit LM. Reprogramming metastatic tumor cells with embryonic microenvironments. Nat Rev Cancer 2007;7:246–255. [PubMed: 17384580]
12. Wanebo HJ, Argiris A, Bergsland E, et al. Targeting growth factors and angiogenesis; using small molecules in malignancy. Cancer Metastasis Rev 2006;25(2):279–292. [PubMed: 16770540]
13. Gille J. Antiangiogenic cancer therapies get their act together: current developments and future prospects of growth factor- and growth factor receptor-targeted approaches. Exp Dermatol 2006;15 (3):175–186. [PubMed: 16480425]
14. Sparano JA, Gray R, Giantonio B, et al. Evaluating antiangiogenesis agents in the clinic: the Eastern Cooperative Oncology Group Portfolio of Clinical Trials. Clin Cancer Res 2004;10(4):1206–1211. [PubMed: 14977816]
15. Motl S. Bevacizumab in combination chemotherapy for colorectal and other cancers. Am J Health Syst Pharm 2005;62(10):1021–1032. [PubMed: 15901587]
16. Vacca A, Ria R, Ribatti D, et al. [Angiogenesis and tumor progression in melanoma]. Recenti Prog Med 2000;91(11):581–587. [PubMed: 11125952]
17. Plum SM, Holaday JW, Ruiz A, et al. Administration of a liposomal FGF-2 peptide vaccine leads to abrogation of FGF-2-mediated angiogenesis and tumor development. Vaccine 2000;19(9–10):1294–1303. [PubMed: 11137269]
18. Sessa C, Vigano L, Grasselli G, et al. Phase I clinical and pharmacological evaluation of the multi-tyrosine kinase inhibitor SU006668 by chronic oral dosing. Eur J Cancer 2006;42(2):171–178. [PubMed: 16406576]
19. Odorisio T, Cianfarani F, Failla CM, et al. The placenta growth factor in skin angiogenesis. J Dermatol Sci 2006;41(1):11–19. [PubMed: 16216466]
20. Donnini S, Machein MR, Plate KH, et al. Expression and localization of placenta growth factor and PIGF receptors in human meningiomas. J Pathol 1999;189(1):66–71. [PubMed: 10451490]
21. Luttun A, Autiero M, Tjwa M, et al. Genetic dissection of tumor angiogenesis: are PIGF and VEGFR-1 novel anti-cancer targets? Biochim Biophys Acta 2004;1654(1):79–94. [PubMed: 14984769]
22. Bar-Eli M. Role of interleukin-8 in tumor growth and metastasis of human melanoma. Pathobiology 1999;67(1):12–18. [PubMed: 9873223]
23. Liu G, Zhang F, Lee J, et al. Selective induction of interleukin-8 expression in metastatic melanoma cells by transforming growth factor-beta 1. Cytokine 2005;31(3):241–249. [PubMed: 15975819]
24. Mahler DA, Huang S, Tabrizi M, et al. Efficacy and safety of a monoclonal antibody recognizing interleukin-8 in COPD: a pilot study. Chest 2004;126(3):926–934. [PubMed: 15364775]
25. Melnikova VO, Bar-Eli M. Bioimmunotherapy for melanoma using fully human antibodies targeting MCAM/MUC18 and IL-8. Pigment Cell Res 2006;19(5):395–405. [PubMed: 16965268]
26. Mueller BM. Different roles for plasminogen activators and metalloproteinases in melanoma metastasis. Curr Top Microbiol Immunol 1996;213(Pt 1):65–80. [PubMed: 8814995]

27. Delbaldo C, Masouye I, Saurat JH, et al. Plasminogen activation in melanocytic neoplasia. Cancer Res 1994;54(16):4547–4552. [PubMed: 8044805]
28. Hearing VJ, Law LW, Corti A, et al. Modulation of metastatic potential by cell surface urokinase of murine melanoma cells. Cancer Res 1988;48(5):1270–1278. [PubMed: 2963689]
29. Min HY, Doyle LV, Vitt CR, et al. Urokinase receptor antagonists inhibit angiogenesis and primary tumor growth in syngeneic mice. Cancer Res 1996;56(10):2428–2433. [PubMed: 8625323]
30. Berkenblit A, Matulonis UA, Kroener JF, et al. A6, a urokinase plasminogen activator (uPA)-derived peptide in patients with advanced gynecologic cancer: a phase I trial. Gynecol Oncol 2005;99(1):50–57. [PubMed: 16023182]
31. Muehlenweg B, Sperl S, Magdolen V, et al. Interference with the urokinase plasminogen activator system: a promising therapy concept for solid tumours. Expert Opin Biol Ther 2001;1(4):683–691. [PubMed: 11727504]
32. Kuphal S, Bauer R, Bosserhoff AK. Integrin signaling in malignant melanoma. Cancer Metastasis Rev 2005;24(2):195–222. [PubMed: 15986132]
33. Hynes RO. The emergence of integrins: a personal and historical perspective. Matrix Biol 2004;23 (6):333–340. [PubMed: 15533754]
34. Eskens FA, Dumez H, Hoekstra R, et al. Phase I and pharmacokinetic study of continuous twice weekly intravenous administration of Cilengitide (EMD 121974), a novel inhibitor of the integrins alphavbeta3 and alphavbeta5 in patients with advanced solid tumours. Eur J Cancer 2003;39(7):917–926. [PubMed: 12706360]
35. Stupack DG, Cheresh DA. Integrins and angiogenesis. Curr Top Dev Biol 2004;64:207–238. [PubMed: 15563949]
36. Seftor RE. Role of the beta3 integrin subunit in human primary melanoma progression: multifunctional activities associated with alpha(v)beta3 integrin expression. Am J Pathol 1998;153 (5):1347–1351. [PubMed: 9811323]
37. Posey JA, Khazaeli MB, DelGrosso A, et al. A pilot trial of Vitaxin, a humanized anti-vitronectin receptor (anti alpha v beta 3) antibody in patients with metastatic cancer. Cancer Biother Radiopharm 2001;16(2):125–132. [PubMed: 11385959]
38. Nikolopoulos SN, Blaikie P, Yoshioka T, et al. Integrin beta4 signaling promotes tumor angiogenesis. Cancer Cell 2004;6(5):471–483. [PubMed: 15542431]
39. Ziober BL, Chen YQ, Ramos DM, et al. Expression of the alpha7beta1 laminin receptor suppresses melanoma growth and metastatic potential. Cell Growth Differ 1999;10(7):479–490. [PubMed: 10437916]
40. Guo W, Giancotti FG. Integrin signalling during tumour progression. Nat Rev Mol Cell Biol 2004;5 (10):816–826. [PubMed: 15459662]
41. Ramakrishnan V, Bhaskar V, Law DA, et al. Preclinical evaluation of an anti-alpha5beta1 integrin antibody as a novel anti-angiogenic agent. J Exp Ther Oncol 2006;5(4):273–286. [PubMed: 17024968]
42. Brown LF, Papadopoulos-Sergiou A, Berse B, et al. Osteopontin expression and distribution in human carcinomas. Am J Pathol 1994;145(3):610–623. [PubMed: 8080043]
43. Geissinger E, Weisser C, Fischer P, et al. Autocrine stimulation by osteopontin contributes to antiapoptotic signalling of melanocytes in dermal collagen. Cancer Res 2002;62(16):4820–4828. [PubMed: 12183442]
44. Reiniger IW, Wolf A, Welge-Lussen U, et al. Osteopontin as a serologic marker for metastatic uveal melanoma: results of a pilot study. Am J Ophthalmol 2007;143(4):705–707. [PubMed: 17386288]
45. Semba T, Funahashi Y, Ono N, et al. An angiogenesis inhibitor E7820 shows broad-spectrum tumor growth inhibition in a xenograft model: possible value of integrin alpha2 on platelets as a biological marker. Clin Cancer Res 2004;10(4):1430–1438. [PubMed: 14977846]
46. Pochec E, Litynska A, Amoresano A, et al. Glycosylation profile of integrin alpha 3 beta 1 changes with melanoma progression. Biochim Biophys Acta 2003;1643(1–3):113–123. [PubMed: 14654234]
47. Miller DH, Khan OA, Sheremata WA, et al. A controlled trial of natalizumab for relapsing multiple sclerosis. N Engl J Med 2003;348(1):15–23. [PubMed: 12510038]

48. Felding-Habermann B, Fransvea E, O'Toole TE, et al. Involvement of tumor cell integrin alpha v beta 3 in hematogenous metastasis of human melanoma cells. Clin Exp Metastasis 2002;19(5):427–436. [PubMed: 12198771]

49. Seftor RE, Seftor EA, Stetler-Stevenson WG, et al. The 72 kDa type IV collagenase is modulated via differential expression of alpha v beta 3 and alpha 5 beta 1 integrins during human melanoma cell invasion. Cancer Res 1993;53(14):3411–3415. [PubMed: 7686818]

50. Tucker GC. Integrins: molecular targets in cancer therapy. Curr Oncol Rep 2006;8(2):96–103. [PubMed: 16507218]

51. Cianfrocca ME, Kimmel KA, Gallo J, et al. Phase 1 trial of the antiangiogenic peptide ATN-161 (Ac-PHSCN-NH(2)), a beta integrin antagonist, in patients with solid tumours. Br J Cancer 2006;94(11): 1621–1626. [PubMed: 16705310]

52. Overall CM, Lopez-Otin C. Strategies for MMP inhibition in cancer: innovations for the post-trial era. Nat Rev Cancer 2002;2(9):657–672. [PubMed: 12209155]

53. Chirco R, Liu XW, Jung KK, et al. Novel functions of TIMPs in cell signaling. Cancer Metastasis Rev 2006;25(1):99–113. [PubMed: 16680576]

54. Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc Res 2006;69(3):562–573. [PubMed: 16405877]

55. Chen WT, Wang JY. Specialized surface protrusions of invasive cells, invadopodia and lamellipodia, have differential MT1-MMP, MMP-2, and TIMP-2 localization. Ann N Y Acad Sci 1999;878:361–371. [PubMed: 10415741]

56. Hofmann UB, Westphal JR, Van Muijen GN, et al. Matrix metalloproteinases in human melanoma. J Invest Dermatol 2000;115(3):337–344. [PubMed: 10951266]

57. Kerkela E, Saarialho-Kere U. Matrix metalloproteinases in tumor progression: focus on basal and squamous cell skin cancer. Exp Dermatol 2003;12(2):109–125. [PubMed: 12702139]

58. Nikkola J, Vihinen P, Vuoristo MS, et al. High serum levels of matrix metalloproteinase-9 and matrix metalloproteinase-1 are associated with rapid progression in patients with metastatic melanoma. Clin Cancer Res 2005;11(14):5158–5166. [PubMed: 16033831]

59. Durko M, Navab R, Shibata HR, et al. Suppression of basement membrane type IV collagen degradation and cell invasion in human melanoma cells expressing an antisense RNA for MMP-1. Biochim Biophys Acta 1997;1356(3):271–280. [PubMed: 9194570]

60. Cotignola J, Reva B, Mitra N, et al. Matrix Metalloproteinase-9 (MMP-9) polymorphisms in patients with cutaneous malignant melanoma. BMC Med Genet 2007;8:10. [PubMed: 17346338]

61. Schnaeker EM, Ossig R, Ludwig T, et al. Microtubule-dependent matrix metalloproteinase-2/matrix metalloproteinase-9 exocytosis: prerequisite in human melanoma cell invasion. Cancer Res 2004;64 (24):8924–8931. [PubMed: 15604254]

62. Wojtowicz-Praga S, Torri J, Johnson M, et al. Phase I trial of Marimastat, a novel matrix metalloproteinase inhibitor, administered orally to patients with advanced lung cancer. J Clin Oncol 1998;16(6):2150–2156. [PubMed: 9626215]

63. Rudek MA, Figg WD, Dyer V, et al. Phase I clinical trial of oral COL-3, a matrix metalloproteinase inhibitor, in patients with refractory metastatic cancer. J Clin Oncol 2001;19(2):584–592. [PubMed: 11208854]

64. Jin X, Yagi M, Akiyama N, et al. Matriptase activates stromelysin (MMP-3) and promotes tumor growth and angiogenesis. Cancer Sci 2006;97(12):1327–1334. [PubMed: 16999819]

65. Wojtowicz-Praga S, Low J, Marshall J, et al. Phase I trial of a novel matrix metalloproteinase inhibitor batimastat (BB-94) in patients with advanced cancer. Invest New Drugs 1996;14(2):193–202. [PubMed: 8913840]

66. Bodey B, Bodey B Jr, Groger AM, et al. Invasion and metastasis: the expression and significance of matrix metalloproteinases in carcinomas of the lung. In Vivo 2001;15(2):175–180. [PubMed: 11317524]

67. Airola K, Karonen T, Vaalamo M, et al. Expression of collagenases-1 and -3 and their inhibitors TIMP-1 and -3 correlates with the level of invasion in malignant melanomas. Br J Cancer 1999;80 (5–6):733–743. [PubMed: 10360651]

68. Hofmann UB, Westphal JR, Zendman AJ, et al. Expression and activation of matrix metalloproteinase-2 (MMP-2) and its co-localization with membrane-type 1 matrix

metalloproteinase (MT1-MMP) correlate with melanoma progression. J Pathol 2000;191(3):245–256. [PubMed: 10878545]

69. Anand-Apte B, Bao L, Smith R, et al. A review of tissue inhibitor of metalloproteinases-3 (TIMP-3) and experimental analysis of its effect on primary tumor growth. Biochem Cell Biol 1996;74(6):853–862. [PubMed: 9164653]
70. Bloomston M, Shafii A, Zervos E, et al. TIMP-1 antisense gene transfection attenuates the invasive potential of pancreatic cancer cells in vitro and inhibits tumor growth in vivo. Am J Surg 2005;189 (6):675–679. [PubMed: 15910719]
71. Pavlaki M, Zucker S. Matrix metalloproteinase inhibitors (MMPIs): the beginning of phase I or the termination of phase III clinical trials. Cancer Metastasis Rev 2003;22(2–3):177–203. [PubMed: 12784996]
72. Coussens LM, Fingleton B, Matrisian LM. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science 2002;295(5564):2387–2392. [PubMed: 11923519]
73. Folkman J. Tumor angiogensis: role in regulation of tumor growth. Symp Soc Dev Biol 1974;30(0): 43–52. [PubMed: 4600889]
74. Jain RK, Duda DG, Clark JW, et al. Lessons from phase III clinical trials on anti-VEGF therapy for cancer. Nat Clin Pract Oncol 2006;3(1):24–40. [PubMed: 16407877]
75. Shih T, Lindley C. Bevacizumab: an angiogenesis inhibitor for the treatment of solid malignancies. Clin Ther 2006;28(11):1779–1802. [PubMed: 17212999]
76. Eisen T, Ahmad T, Flaherty KT, et al. Sorafenib in advanced melanoma: a Phase II randomised discontinuation trial analysis. Br J Cancer 2006;95(5):581–586. [PubMed: 16880785]
77. Rudge JS, Thurston G, Davis S, et al. VEGF trap as a novel antiangiogenic treatment currently in clinical trials for cancer and eye diseases, and VelociGene- based discovery of the next generation of angiogenesis targets. Cold Spring Harb Symp Quant Biol 2005;70:411–418. [PubMed: 16869778]
78. Mita MM, Rowinsky EK, Forero L, et al. A phase II, pharmacokinetic, and biologic study of semaxanib and thalidomide in patients with metastatic melanoma. Cancer Chemother Pharmacol 2007;59(2):165–174. [PubMed: 16736151]
79. Rasmussen HS, McCann PP. Matrix metalloproteinase inhibition as a novel anticancer strategy: a review with special focus on batimastat and marimastat. Pharmacol Ther 1997;75(1):69–75. [PubMed: 9364582]
80. Quirt I, Bodurth A, Lohmann R, et al. Phase II study of marimastat (BB-2516) in malignant melanoma: a clinical and tumor biopsy study of the National Cancer Institute of Canada Clinical Trials Group. Invest New Drugs 2002;20(4):431–437. [PubMed: 12448662]
81. Lee L, Sharma S, Morgan B, et al. Biomarkers for assessment of pharmacologic activity for a vascular endothelial growth factor (VEGF) receptor inhibitor, PTK787/ZK 222584 (PTK/ZK): translation of biological activity in a mouse melanoma metastasis model to phase I studies in patients with advanced colorectal cancer with liver metastases. Cancer Chemother Pharmacol 2006;57(6):761–771. [PubMed: 16172907]
82. Basche M, Gustafson DL, Holden SN, et al. A phase I biological and pharmacologic study of the heparanase inhibitor PI-88 in patients with advanced solid tumors. Clin Cancer Res 2006;12(18): 5471–5480. [PubMed: 17000682]
83. Miller DW, Vosseler S, Mirancea N, et al. Rapid vessel regression, protease inhibition, and stromal normalization upon short-term vascular endothelial growth factor receptor 2 inhibition in skin carcinoma heterotransplants. Am J Pathol 2005;167(5):1389–1403. [PubMed: 16251423]
84. Trikha M, Zhou Z, Nemeth JA, et al. CNTO 95, a fully human monoclonal antibody that inhibits alphav integrins, has antitumor and antiangiogenic activity in vivo. Int J Cancer 2004;110(3):326–335. [PubMed: 15095296]
85. Gogas H, Polyzos A, Stavrinidis I, et al. Temozolomide in combination with celecoxib in patients with advanced melanoma. A phase II study of the Hellenic Cooperative Oncology Group. Ann Oncol 2006;17(12):1835–1841. [PubMed: 16980601]
86. Willett CG, Boucher Y, di Tomaso E, et al. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat Med 2004;10(2):145–147. [PubMed: 14745444]

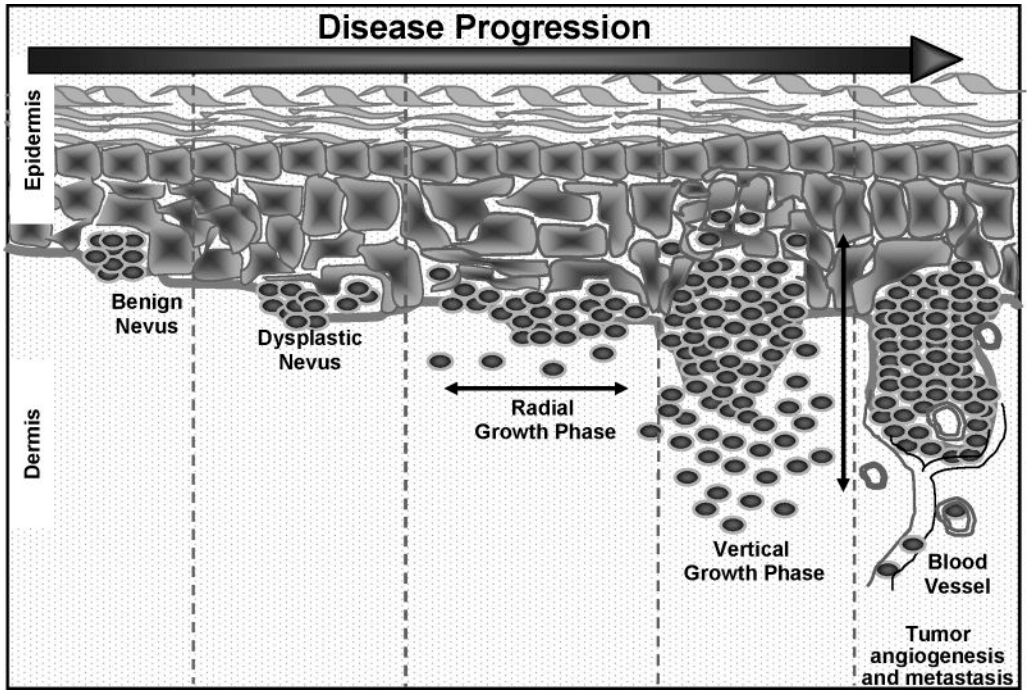


**Figure 1. Malignant progression of melanoma**

Aberrant proliferation of normal melanocytes leads to formation of benign naevus. The rapidly proliferating transformed melanocytes form dysplastic naevi, which are asymmetric with irregular borders. This is followed by a rapid radial growth phase, which is characterized by intraepidermal growth. The last step in benign melanoma growth is the vertical growth phase. It is also called the dermal invasion phase. Malignant melanomas develop vasculature very rapidly providing a route for further metastasis.

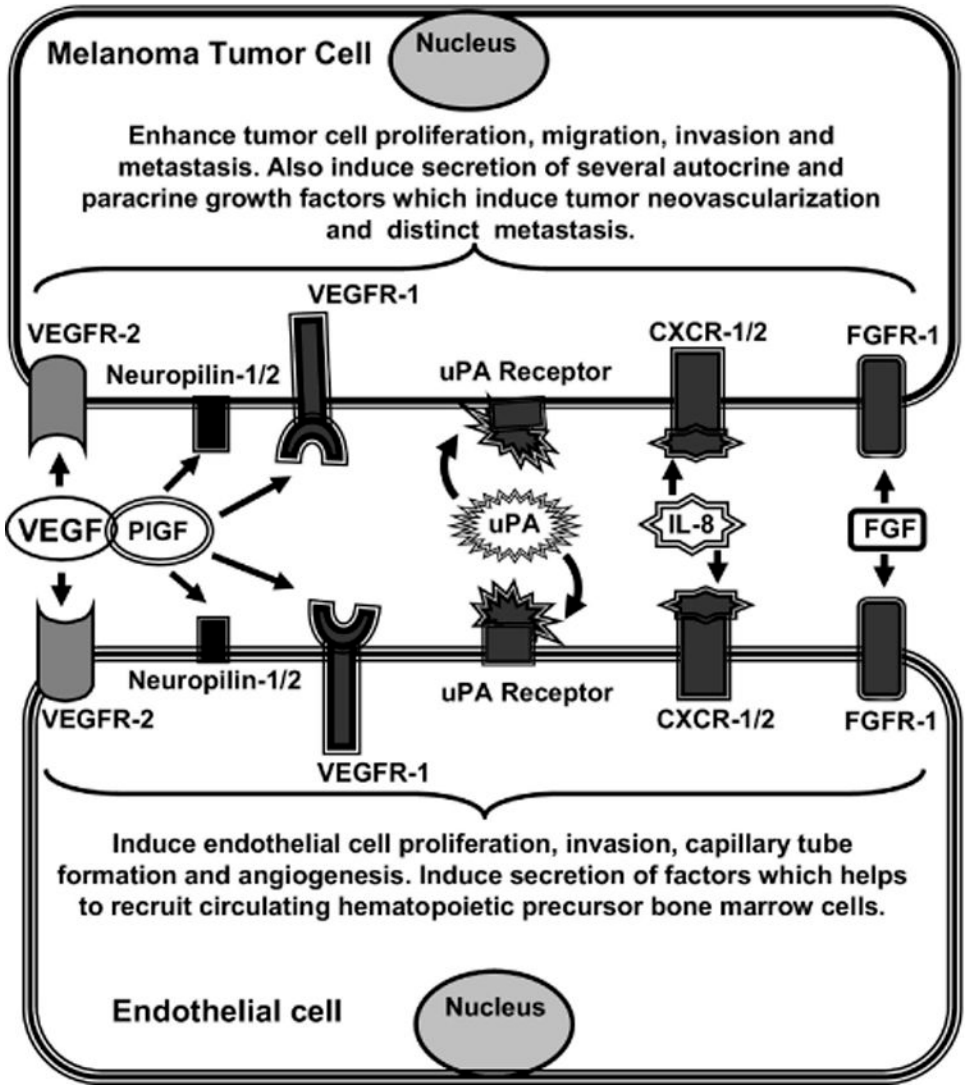


**Figure 2. Cell surface receptor mimicry between malignant melanoma cells and endothelial cells**

Malignant melanoma cells express VEGFR-2, VEGFR-1 and co-receptor neuropilin-1/2 which are commonly not expressed on most of the cancer cells. Therefore, VEGF is able to induce similar intracellular signaling responses in both endothelial and melanoma cells. Placental growth factor can independently bind to VEGFR-1 and neuropilin-1/2 to induce intracellular signaling. PIGF also form heterodimeric complexes with VEGF and interact with VEGFR-2. Other receptors expressed on both melanoma and endothelial cells include uPA receptor, CXCR-1/2 and FGFR-1. Therefore, there is a spectrum of growth factors/chemokines which are able to induce similar signaling responses in both cell types.

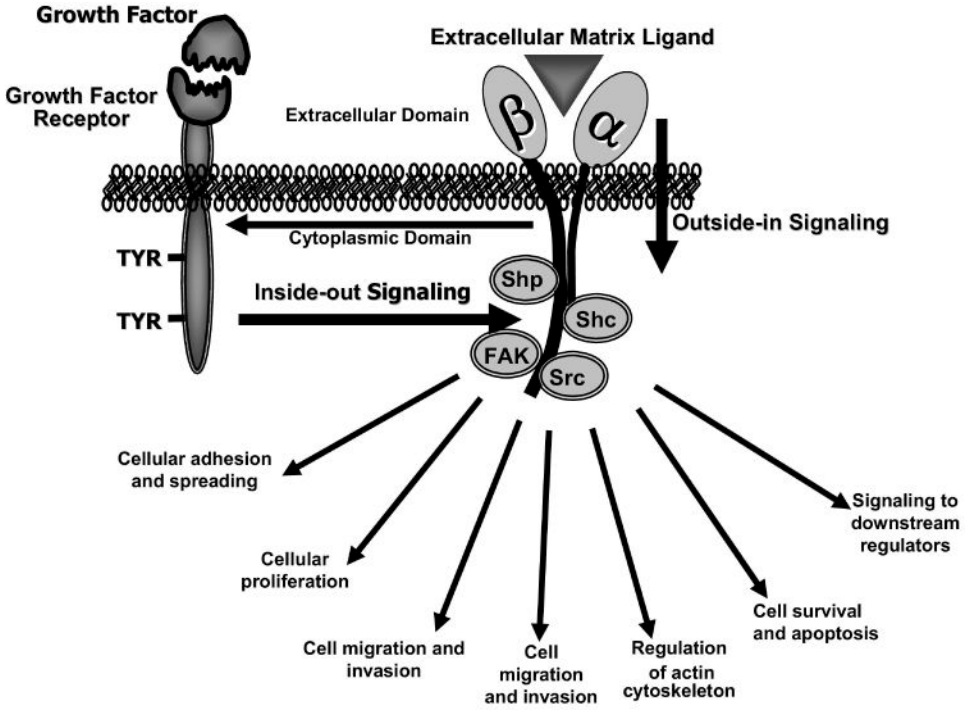


**Figure 3. Regulation of integrin signaling by ECM proteins and growth factor receptors**

Integrins and their ligand expression are up-regulated in most of the human melanomas. Ligand binding to the extracellular domain of specific integrin leads to initiation of signaling events required for a broad spectrum of processes such as cellular proliferation, survival, and actin cytoskeleton regulation. Integrin interactions with its ligands are the prerequisites for cellular adhesion, invasion and migration. Growth factors, via receptors expressed on endothelial cells or melanoma cells, are able to regulate integrin function activity, i.e. its affinity and avidity of interactions with ECM proteins. The process of functional crosstalk between growth factor receptors and integrins is regulated by several intracellular signaling molecules which are able to shuffle between growth factor receptors and integrin cytoplasmic domains.

**Table 1**

Major growth factors expressed in malignant melanoma and clinical significance

| Molecule | Functions | Clinical Trial | Reference |
|---|---|---|---|
| VEGF/VEGFR | Enhance EC proliferation, migration and angiogenesis | Phase 2/3 | [14], [15] |
| FGF/FGFR | Enhance expression of VEGF and uPA and prevent EC apoptosis | Phase 1 | [17], [18] |
| PIGF | Recruit bone marrow-derived hematopoietic precursors site of angiogenesis | None | [19], [20] |
| IL-8 | Induce tumor neovascularization and tumor cell proliferation | Phase 2 | [24], [25] |
| uPA | Increase tumor vasculature and migration and metastasis of tumor cells | Phase 1 | [30], [31] |

**Table 2**

Major integrin receptors expressed on endothelial/malignant melanoma cells and clinical significance

| Integrin Receptor | Functions | Expression | Clinical Trial | Reference |
|---|---|---|---|---|
| $\alpha_v\beta_3$ | Enhance tumor vasculature and tumor growth | EC, MM | Phase 2 | [36] |
| $\alpha_v\beta_5$ | Similar to $\alpha_v\beta_3$ integrin induce tumor growth and angiogenesis | EC | Phase 2 | [34] |
| $\alpha_5\beta_1$ | Essential for metastasis of melanoma cells | EC, MM | Phase 2 | [41] |
| $\alpha_6\beta_4$ | Required for vascular maturation, associated with aggressive behavior of tumor cells | EC, MM | None | [38] |
| $\alpha_2\beta_1$ | Essential for endothelial and tumor cell interaction with basal lamina | EC, MM | Phase 1 | [45] |
| $\alpha_3\beta_1$ | Required for malignant progression of melanoma cells | MM | None | [46] |
| $\alpha_4\beta_1$ | Required for melanoma cell metastasis | EC, MM | Phase 1/2 | [47] |

**Table 3**

Major MMPs expressed in malignant melanoma and clinical significance

| MMP | Functions | Clinical Trial | Reference |
|---|---|---|---|
| MMP-1 | Collagen type I and IV degradation and melanoma cell invasion | Phase 2/3 | [59] |
| MMP-2 | Melanoma cell invasion and angiogenesis | Phase 2/3 | [63] |
| MMP-3 | Promotes tumor growth and angiogenesis | Phase 2/3 | [64] |
| MMP-7 | Growth, invasion, metastasis of tumor cells and angiogenesis | Phase 1/2 | [65] |
| MMP-9 | Tumor cell invasion, metastasis and angiogenesis | Phase 1 to 3 | [62] |
| MMP-10 | Malignant melanoma tumor cell invasion and metastasis | None | [66] |
| MMP-13 | Expression correlates with tumor progression and aggressive tumor behavior | None | [67] |
| MMP-14 | Melanoma tumor cell invasion and angiogenesis | None | [68] |

**Table 4**

Major angiogenic molecules targeted for cancer therapy, clinical approaches and outcome.

| Category | Molecular Target | Class | Name | Preclinical studies | Clinical trials status in melanoma or other cancers | Some observed side effects | Refs |
|---|---|---|---|---|---|---|---|
| **Angiogenic growth factors and its receptors** | VEGF | Monoclonal antibody | Bevacizumab | Very potent inhibitor of melanomas in several in vivo models | Phase II (response observed) | Minimal toxicity, delayed wound healing | 75 |
| | Tyrosine kinase receptors | Small molecule | Sorafenib | Inhibitor of various tumors in vivo and in vitro | Phase II ( minimal effect as a single agent) | Well tolerated | 76 |
| | VEGF | Soluble VEGF receptor | VEGF-trap | Potent inhibitor in various in vivo models | Phase I/II | Same as above | 77 |
| | VEGF receptor-1 | Small molecule | Semaxinib (SU-5416) | Effective in vivo | Phase II (partial response observed) | Deep vein thrombosis | 78 |
| | VEGF receptors | Small molecule | PTK/ZK | Effective in vivo (including melanomas) | Phase I/II | Well tolerated | 81 |
| | Heparanase inhibitor (VEGF and FGF) | Mixture of oligosaccharides | PI-88 | Inhibitor of VEGF/ FGF release and tumor growth and invasion in vivo | Phase I (partial response, recommended for phase II) | Well tolerated, thrombocytopenia | 82 |
| | VEGF receptor-2 | Monoclonal antibody | DC101 | Very potent in vivo | -- | -- | 83 |
| **Receptors for extracellular matrix, integrins** | Integrin $\alpha v\beta_3$ | Monoclonal antibody | Vitaxin (MEDI-522) | Potent in vivo inhibitor of tumor growth and metastasis | Phase I (recommended for phase II) | Limited toxicity (MI) | 37 |
| | αv integrins | Monoclonal antibody | CNTO95 | Same | Phase I/II | Well tolerated | 84 |
| | alphavbeta3/alphav beta5 | Small molecule | Cilengitide (EMD 121974) | Same | Phase I/II | Well tolerated | 34 |
| **Components of extracellular matrix and proteases** | MMPs | Small molecule | Batimastat | Inhibits melanoma growth and lung metastasis in mice | Phase I/II, limited activity | Poor solubility | 79 |
| | MMPs | Small molecule | Marimastat | Inhibits melanoma growth in vivo | Phase I/I | Muscle pain and stiffness | 80 |
| | Extracellular matrix | Fragment of matrix | Endostatin | Potent angiogenesis inhibitor in vitro and in vivo | Phase I | Minimal toxicity | |
| **Complex mechanism of action** | Angiogenesis inhibitors and immunomodulators | Small molecule | Thalidomide and lenalidomide | Effective in vivo | Phase II | Neuropathy, skin rash | 78 |
| | Cyclooxygenase-2 | Small molecule | Celecoxib | Effective in vivo | Phase I/II (shows response) | Well tolerated | 85 |