We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

8

Pigment Epithelium-Derived Factor – An Angiostatic Factor with a Broader Function in Melanoma

A. Fernández-Barral[1], J.L. Orgaz[1,2] and B. Jiménez[1]
[1]*Instituto de Investigaciones Biomédicas, CSIC-UAM-Departamento de Bioquímica UAM, Madrid,*
[2]*Present address: Randall Division of Cell & Molecular Biophysics, King's College London, London, SE1 1UL,*
[1]*Spain*
[2]*U.K.*

1. Introduction

Metastatic spread is achieved through changes in the tissue microenvironment driven by tumor cells that allow the formation of various dissemination routes using a variety of mechanisms; such as angiogenesis and vasculogenesis (hematogeneous routes), lymphangiogenesis (lymphatic routes), and in some particular cases like melanoma, vasculogenic mimicry (vasculogenic channels lined by melanoma cells) (Carmeliet, 2005; Hendrix *et al*., 2003; Kopp *et al*., 2006; Tammela and Alitalo, 2010). Building of dissemination routes has to be coordinated with the acquisition of new capabilities by tumor cells that enable them to locally invade, intravasate into dissemination channels, survive in the circulation, extravasate, and ultimately adapt to a foreign territory. All this complex cascade of events is orchestrated by multiple cell types and diverse families of factors and signaling circuits controlling intracellular as well as intercellular key communication events (Nguyen *et al*., 2009b).

Interestingly, a particular subset of extracellular factors have the dual capacity to simultaneously impinge on the formation of the dissemination routes and to modulate many of the properties that the tumor cells themselves have to acquire in order to fulfill all steps required to successfully colonize a foreign territory starting from a primary lesion in a drastically different environment. This chapter focuses on an angiostatic factor, pigment epithelium derived factor (PEDF), with a broader function in melanoma that allows it to dually impinge on destroying some of the more relevant dissemination routes and on counteracting key tumor cell properties that enable the metastatic spread of melanoma cells. Understanding of the molecular and cellular mechanisms controlling melanoma progression has become an active field of research over the last five years unveiling a complex intertwined relationship between melanoma cells and the diverse cell types present in the tumor microenvironment, as well as a number of key molecular mediators (Shackleton and Quintana, 2010; Villanueva and Herlyn, 2008). Plasticity of melanoma cells allows them for appropriate reprogramming underlying the decision making process that arbitrates

proliferation and migration as mutually exclusive cellular responses that need to alternate in the course of tumor progression (Hendrix *et al.*, 2007; Hoek *et al.*, 2008). We have recently interrogated the role of PEDF, an angiostatic factor produced at high levels by skin melanocytes, in controlling the switch between proliferative and invasive states of melanoma cells and its contribution to restrict the metastatic cascade. Our results demonstrate that loss of PEDF expression enables melanoma cells to acquire an invasive state and therefore its reprogramming is critical for the malignant progression of melanoma (Orgaz *et al.*, 2009).

2. Angiogenesis and melanoma. The role of endogenous inhibitors of angiogenesis

As in every tissue of our body angiogenesis is finely tuned in the skin by the balance of endogenous angiogenic growth factors and endogenous angiogenic inhibitors (Jimenez and Volpert, 2001). Although the pattern of skin vascularization established during development renders the skin a mildly hypoxic microenvironment (pO_2 in the dermal/epidermal junctions ranging from 0.5% to 10%) (Bedogni and Powell, 2009), a number of physiological processes like skin wound healing and cycles of hair follicle growth, and an increasingly recognized number of cutaneous pathologies (Laquer *et al.*, 2009; Nguyen *et al.*, 2009a) require new vessel formation to respectively achieve proper tissue homeostasis in physiological contexts or to chronically activate angiogenesis in the pathological settings.

Angiogenesis is a hallmark of cancer and it is of significant relevance for the life threatening stage of the disease, the metastatic spread (Hanahan and Weinberg, 2000; Hanahan and Weinberg, 2011). Angiogenesis is a pivotal process required to effectively deliver oxygen and nutrients and to eliminate waste products in lesions beyond 1-2mm of diameter (Folkman, 2006). Unlimited growth of primary lesions, activation of dormant micrometastases (Goss and Chambers, 2010), as well as the growth of micrometastases to macrometastases (Gao *et al.*, 2008), all require neovascularization. The so called tumor angiogenic switch refers to the mechanisms responsible for shifting the balance toward predominance of angiogenic growth factors accompanied by loss of angiogenesis inhibitors. Activation of tumor angiogenic switch triggers the transition from the avascular to the vascular phase of tumor growth, which is characterized by uncontrolled, excessive and aberrant neovascularization. The vascular phase sustains unlimited neoplastic growth and provides diverse vascular routes for the metastatic dissemination of the primary lesion. There is significant evidence supporting that cancer metastasis can be determined by the angiogenic potential of the primary tumor cells (Kerbel, 2008). Also, preclinical studies using mouse models, as well as clinical studies using biopsies have shown that there is a direct association between incidence of metastases and the microvascular density in vascular hot spots in the tumor periphery (Nico *et al.*, 2008).

Based on the relevance of tumor neovascularization for the progression of the disease and patient outcome, there has been over the last four decades an explosion of the cellular and molecular knowledge of the mechanisms and key molecules involved in the creation of the diverse types of vessels networks that allow for tumor cell dissemination. All this knowledge also led to a rapid and fruitful translation to the clinic of the first generation of antiangiogenic drugs (Ellis and Hicklin, 2008; Ferrara and Kerbel, 2005; Jain, 2008; Jubb *et al.*, 2006; Loges *et al.*, 2009; Orgaz *et al.*, 2008) which have been used up to now in the context of advanced disease and as a general rule in combination with a wide range of

chemotherapeutic agents or radiation therapy. Also, the general principle of antiangiogenic therapy of cancer in the clinic has been almost exclusively based on the use of single antiangiogenic agents or drugs. Although most of these studies have obtained reasonably encouraging results, further evaluation of more complex therapeutic regimens targeting simultaneously multiple angiogenesis pathways should be warranted in patients with advanced melanoma and other cancers; or when required to overcome resistance to first line antiangiogenic drugs (Bergers and Hanahan, 2008). Notwithstanding, the broad armory of identified antiangiogenic drugs should allow designing optimum combinations of antiangiogenic drugs that hopefully will be: (i) more efficient and of greater benefit for each particular type of cancer, (ii) used as second line antiangiogenic therapies in cases of resistance to first line antiangiogenic drugs, or (iii) useful to suitably design the most likely effective strategy considering the characteristics of each patient's type of tumor vascular bed.

The mildly hypoxic microenvironment of the skin significantly contributes to melanocyte transformation, as the result of hypoxia effects promoting both proliferation and survival. Thus, hypoxia has emerged as a relevant tumor-promoting environmental factor in melanoma (Bedogni and Powell, 2009). Additionally, hypoxia is one of the main regulators of angiogenic growth factors and angiogenic inhibitors, which contributes to tilt the balance toward inducers of angiogenesis and to impose the loss of relevant angiostatic factors during tumor progression (Rey and Semenza, 2010).

Studies with human melanoma xenograft models in nude mice as well as with human melanoma biopsies demonstrated that melanoma cells are an important source of angiogenic growth factors like vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), placenta growth factor (PlGF), transforming growth factors-α and β (TGF- α and β), platelet derived growth factor-B (PDGF-B) and interleukin-8 (IL-8), among others (Basu *et al*., 2009; Mahabeleshwar and Byzova, 2007). A number of studies have correlated melanoma neovascularization with poor patient prognosis, overall survival, ulceration and increase rate of relapse; as it is the case in many other types of cancers (Ria *et al*., 2010). However, production of angiogenesis inhibitors by melanoma cells and their regulation in the course of melanoma progression has remained poorly explored.

We have recently focused on the study of the angiostatic factor pigment epithelium derived factor (PEDF) in human melanocytes and melanoma progression (Fernandez-Garcia *et al*., 2007; Garcia *et al*., 2004; Orgaz *et al*., 2009). PEDF was originally described as the most potent angiostatic factor in the eye that plays a relevant role in ensuring the correct pattern of vascularization of diverse eye compartments (Bouck, 2002). PEDF is produced at high levels by retinal pigment epithelial (RPE) cells, and it counteracts a number of potent angiogenic growth factors in the retina like VEGF, insulin growth factor-1 (IGF-1), bFGF, etc; ensuring the right balance of angiogenic regulators that leads to an optimum physiological pattern of blood vessels for correct retinal function. Importantly, avascular eye compartments like the vitreous and cornea are rich in PEDF. To further support the relevance of PEDF in the eye, a number of eye pathologies like diabetic retinopathy and eye related macular degeneration are associated with loss of PEDF expression and therefore predominance of the action of angiogenic growth factors, leading to excessive and aberrant vascularization patterns associated with loss of vision (Tombran-Tink, 2010).

We have recently shown that melanocytes are also among the cell types in our body that produce and secrete the highest levels of PEDF (Orgaz *et al*., 2009), which are comparable to the levels produced by RPE cells, neural cells or retinoblastoma cells. However, endothelial

cells, one of the main targets of PEDF's action, produce very low levels of this angiostatic factor and therefore rely on other cell types to bring PEDF to many scenarios where proper tissue homeostasis requires halting the angiogenic cascade to render the vasculature to a quiescent state. If PEDF is highly produced by melanocytes the following questions arise: (i) is there an autocrine role of PEDF on pigment cells? It has been recently described that PEDF is stored in melanosomes, although its putative role in the regulation of pigment production and secretion remains to be explored (Chi *et al.*, 2006). Furthermore, PEDF directly modulates the proliferative and migratory capability of normal melanocytes (Orgaz *et al.*, 2009), (ii) is PEDF expression regulated during melanoma progression?, and which are the functional consequences of its modulation? Our insights about these questions will be addressed in the following section.

Primary melanoma biopsies are characterized by high PEDF expression in the vast majority of human biopsies analyzed, although a significant degree of heterogeneity exists (Orgaz *et al.*, 2009; and unpublished data). Conversely, PEDF expression is lost in cutaneous metastases of human melanoma (Orgaz *et al.*, 2009).

When is angiogenesis switched on during melanoma progression and its relevance for the metastatic spread of human melanoma is still a matter of certain debate (Basu *et al.*, 2009; Helfrich *et al.*, 2010). Lack of consensus most probably reflects difficulty on adequately defining staging and progression of melanoma, together with limitations of currently available models to explain how melanoma evolves and malignizes (see Section 3.1). It seems plausible that acquisition of angiogenic potential and increase in microvascular density occur gradually as melanoma lesions progress from the radial growth phase (RGP) to the vertical growth phase (VGP) and to the metastatic phase (M). In our studies we found that the most dramatic regulation of PEDF levels corresponds to the transition from primary melanoma to cutaneous metastases of melanoma (described in more detail in Section 3.3). Also most likely melanomas progressively develop a more profuse network of blood vessels from RGP to VGP, but due to the extreme heterogeneity of human melanoma biopsies we were unable to find important differences on the level of PEDF when comparing RGP to VGP biopsies, although there was a tendency to a decrease from RGP to VGP (unpublished data).

We have directly explored by means of global gene expression analysis using microarrays the consequences of PEDF overexpression on the angiogenic potential of human melanoma cells. Importantly, exogenous PEDF overexpression abrogates the ability of aggressive melanoma cells to produce potent angiogenic growth factors like VEGF or IL-8, tilting the balance toward inhibition of melanoma angiogenesis (Orgaz *et al.*, 2011). Given the role of VEGF and IL-8 as highly potent angiogenic growth factors in melanoma, together with their role in directly promoting melanoma cell migration and invasion; the abrogation of their production by melanoma cells upon PEDF's action is of special relevance. Additionally, both VEGF and IL-8 increase vascular permeability and therefore, by eliminating these angiogenic growth factors PEDF normalizes to a certain extent tumor vascular leakiness and thereby impedes melanoma intravasation/extravasation. Finally, a number of extracellular matrix proteins (like collagen IV A2 (COL4A2) and fibronectin 1 (FN1)), matrix enzymes and matrix metalloproteinases (such as tissue inhibitors of metalloproteinases (TIMPs), a disintegrin and metalloproteinase domain (ADAMs), a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif (ADAMTs)) or serine (or cysteine) proteinase inhibitor (SERPINs) and integrins (like integrin β3) relevant in different steps of the angiogenic cascade were modulated by PEDF, with a trend corresponding to halting the angiogenic process (Orgaz *et al.*, 2011).

Finally, as melanoma cells become more aggressive they acquire the ability to mimic molecularly and functionally the endothelial cells. This specific reprogramming, characteristic of highly aggressive melanoma cells, is called vasculogenic mimicry, and endows them with the ability to form vascular channels lined by melanoma cells; which represent a unique escape route for the spread of melanoma (Hess *et al.*, 2007). We found that PEDF is able to impede the formation of melanoma vasculogenic channels in the lungs of nude mice in colonization assays of human melanoma cell lines (Orgaz *et al.*, 2009); which adds another mechanism of antimetastatic action for this multifunctional factor. The molecular basis by which PEDF abrogates melanoma vasculogenic mimicry are still unknown and would be extremely interesting to explore in view of the relevance of this mechanism in the context of melanoma dissemination.

Another relevant aspect of the tumor vasculature that distinguishes it from normal vasculature is the degree of vessel maturation (Baluk *et al.*, 2005; Jain, 2003). Over the last decades it was demonstrated that the efficacy of antiangiogenic strategies in solid tumors is inversely correlated to the degree of maturation of the tumor vascular bed (Helfrich and Schadendorf, 2011; Jimenez and Volpert, 2001). This was the case not only for strategies based on interference with angiogenic growth factors and their receptors, but also for angiostatic factors like PEDF. We demonstrated that PEDF only induces vessel regression of immature vessels poorly covered by pericytes, while those tumor vessels that are covered with pericytes over the course of time of tumor establishment remain invulnerable to PEDF's angiostatic action (Garcia *et al.*, 2004). This important mechanistic observation opened up the following therapeutic windows: (i) use of antiangiogenic strategies at very early stages of tumor progression, when presumably a larger proportion of the vessels are still immature; (ii) use of antiangiogenic strategies in tumor types in which the majority of vessels remain immature; and (iii) design combination therapies in which antiangiogenic strategies directed to endothelial cells are concomitantly used with drugs directed to pericytes aimed to destabilize the tumor vasculature by depriving it of the interactions with pericytes, and therefore rendering it immature. Notwithstanding the limitation on the angiostatic action of PEDF, which is restricted to immature vessels, all direct PEDF's effects on melanoma cells contribute to halt melanoma metastases, and strongly support the development of novel therapeutic strategies based on knowledge of the diverse actions of this biological modifier of melanoma.

The mechanism by which PEDF induces immature blood vessel regression has been studied in detail over the last years by us and others. A common characteristic in the mechanism of action of PEDF and all the endogenous inhibitors of angiogenesis so far described (thrombospondin-1, angiostatin, endostatin, canstatin, tumstatin, etc) is that they are capable of inducing apoptosis in endothelial cells present in immature, remodeling vessels, thus causing selective regression of the expanding aberrant tumor vasculature without affecting normal vessels (Jimenez and Volpert, 2001; Volpert, 2000). Angiogenic growth factors like VEGF act like essential survival factors in this pathological scenario, but the action of endogenous inhibitors of angiogenesis is dominant over that of inducers. PEDF induces apoptosis in remodeling endothelial cells by inducing Fas ligand (CD95L) expression on the surface of activated endothelial cells via nuclear factor kappa-light-chain-enhancer of activated B cells (NFKB) (Aurora *et al.*, 2010; Volpert *et al.*, 2002), which initiates an extrinsic cell death cascade. In concert, PEDF hampers endothelial cells survival by reducing the expression of pro-survival factor caspase-8 FLICE-inhibitory protein (c-FLIP) through the Nuclear Factor of Activated T-cells (NFAT) (Zaichuk *et al.*, 2004). Besides, we have also

demonstrated that under stress conditions like serum withdrawal or lack of extracellular matrix attachment, PEDF has a certain capacity to induce melanoma cell apoptosis although with a weaker potency than in endothelial cells (Garcia *et al.*, 2004). Reduced survival of melanoma cells caused by the absence of attachment in the presence of high PEDF circulating levels has important consequences for the outcome of tumor cells in transit to the metastatic site.

There are still a number PEDF's putative actions on the various tumor vascularization mechanisms described so far that remain to be explored and that are of critical importance: (i) does PEDF affect haematopoyetic precursor cells recruitment?, (ii) does PEDF block vasculogenesis (formation of new vessels from endothelial progenitor cells)?, (iii) does PEDF induce lymphatic vessels regression? There is no report linking PEDF to vasculogenesis, but it has recently been described that PEDF decreases lymph node metastasis of prostate cancer, while paradoxically increases extratumoral lymphangiogenesis by unknown mechanisms (Halin *et al.*, 2010).

In the context of spread of the disease an attractive benefit of antiangiogenic strategies is their potential to keep micrometastases in a dormant state (Gao *et al.*, 2008; Goss and Chambers, 2010). Thrombospondin-1 has been proven to be effective in preventing the growth of dormant pulmonary micrometastases in human melanoma xenografts after surgical resection or curative radiation of the primary tumor (Rofstad *et al.*, 2004; Rofstad *et al.*, 2003). PEDF's capability to maintain dormant micrometastases in check, preventing their growth to macrometastases remains to be evaluated, but it could be of relevant therapeutic interest.

Moreover, it has been shown that PEDF is also produced by a wide variety of epithelial cell types in different tissues and its role in controlling primary tumor growth, angiogenesis and metastatic spread has been explored in a wide range of tumors using diverse mouse models, and analysis of human biopsies. Levels of angiostatic PEDF decrease during the progression of a number of cancers, such as hepatocelular carcinoma (Matsumoto *et al.*, 2004), prostate cancer (Halin *et al.*, 2004; Qingyi *et al.*, 2009), breast adenocarcinoma (Cai *et al.*, 2006), glioblastoma (Guan *et al.*, 2003) and Wilm´s tumors (Abramson *et al.*, 2003). A number of recent excellent reviews cover the antitumor and antimetastatic action of PEDF on several tumor types (Broadhead *et al.*, 2009; Fernandez-Garcia *et al.*, 2007; Hoshina *et al.*, 2010).

3. Halting melanoma progression. The role of pigment epithelium-derived factor

3.1 Cutaneous melanoma. Current models of melanoma progression

Melanoma cells arise from melanocytes, normal cells specialized in the production of pigment melanin, which reside in the basal layer of the epidermis, among other locations, and whose homeostasis is tightly controlled by epidermal keratinocytes (Gray-Schopfer *et al.*, 2007).The classical model of melanoma progression describes melanoma development as a series of histopathological steps regarding the thickness and the grade of invasion of the lesion (Clark, 1991). Nevi are relatively benign lesions that rarely progress to melanoma, in part because most of melanocytes in nevocytic lesions are in a state of senescence-associated growth arrest (Michaloglou *et al.*, 2005). RGP, melanomas have high proliferative potential but null or very low invasive ability, being confined to the epidermis (Gray-Schopfer *et al.*, 2007). In contrast, VGP, melanomas are competent for metastasis, fully invade the upper part of the epidermis as well as the dermis and the subcutaneous tissue, being able to reach

blood or lymph circulation and eventually colonize and develop secondary tumors at distant organs (Chin *et al.*, 2006; Gray-Schopfer *et al.*, 2007). This model is in agreement with the tumor clonal evolution model (Nowell, 1976), which hypothesizes that cancer evolves from a poorly to a highly metastatic phase through accumulation of molecular alterations (mutations and/or epigenetic changes) that enhance proliferative and invasive potential of tumor cells, and that would promote lineal progression from RGP to VGP to metastasis (Miller and Mihm, 2006).

Even though the classification of melanomas according to the thickness of the lesion is one of the most widely used methods for the diagnosis and prognosis of melanoma (Fecher *et al.*, 2007), frequently there are lesions whose thickness does not correlate with their actual aggressiveness and metastatic outcome (Lomuto *et al.*, 2004; Slingluff *et al.*, 1988). Besides, this model does not offer either convincing explanations for the heterogeneity found in metastatic melanoma cells, nor for the persistent failure of anti-melanoma therapies. Consequently, in the last decade researchers have aimed to establish a molecular classification of melanoma utilizing gene expression profiling tools (Fecher *et al.*, 2007; Hoek, 2007; Hoek, 2009). A number of studies have been able to classify collections of melanoma cell lines or biopsies into groups differing in their aggressiveness and metastatic potential, confirming the large heterogeneity of melanoma and the importance of the microenvironment in determining gene expression programs of melanoma cells and progression to metastasis (Bittner *et al.*, 2000; Haqq *et al.*, 2005; Hoek, 2007; Pavey *et al.*, 2004).

One of these studies, by means of genome-wide gene expression analysis and functional assays, described that most melanoma cell lines could be categorized according to their gene expression profile into two extreme phenotypes, proliferative or invasive (Hoek *et al.*, 2006). The proliferative gene signature encompasses a number of melanocytic lineage genes such as microphthalmia-associated transcription factor (MITF) and some of its targets, while the invasive phenotype signature is defined by suppressed expression of proliferative genes in favor of others related to the modification of the tumor microenvironment (Hoek *et al.*, 2006). Based on this and other studies a new melanoma progression model was proposed, which takes into account the heterogeneous nature of melanoma and the key role of the tumor microenvironment (Carreira *et al.*, 2006; Goodall *et al.*, 2008; Hoek, 2009; Hoek *et al.*, 2008; Hoek *et al.*, 2006). This new model, referred to as phenotype switching, considers metastatic potential split into two mutually exclusive and reversible states, proliferative and invasive, and hypothesizes that melanoma progression is driven by the reversible switching between these two phenotypes (Hoek, 2009). A primary lesion would be initially composed of proliferative phenotype cells. Signals from the microenvironment, such as hypoxia or inflammation, would make some cells switch their gene signature to become invasive, which would allow them to escape from the primary tumor and eventually reach and colonize a foreign distant organ. There, signals from the new environment would reprogram cells back to the proliferative signature, ultimately developing metastases (Carreira *et al.*, 2006; Goodall *et al.*, 2008; Hoek *et al.*, 2008).

The phenotype switching model also relies on human melanoma high plasticity and ability to be reprogrammed. As mentioned before, an example of the high plasticity of melanoma cells is vasculogenic mimicry (Seftor *et al.*, 2002). Some aggressive melanoma cells have been found to express molecular markers typical of other cell types, such as endothelial cells, which presumably allow melanoma cells to form vasculogenic networks that mimic blood vessels, and that could serve as an alternative route of escape for melanoma cells. This phenomenon has also been reported to occur *in vivo* in aggressive human melanoma

samples, and correlates with poor outcome (Folberg *et al.*, 2000; Hendrix *et al.*, 2003; Maniotis *et al.*, 1999). Furthermore, poorly aggressive melanoma cells can be reprogrammed to highly aggressive and invasive by exposing them to matrices preconditioned by more aggressive melanoma cells, highlighting the importance of the tumor microenvironment in driving melanoma progression (Postovit *et al.*, 2006; Seftor *et al.*, 2006).

3.2 Melanoma invasion

Primary melanoma patients can be usually cured by surgical removal of the tumor when detected early, but most of the times melanoma rapidly metastasizes with a poor outcome (Gray-Schopfer *et al.*, 2007). Therefore it is essential to understand the mechanisms by which melanoma cells escape from the primary tumor and spread and invade other organs, in order to develop improved therapies that increase patient survival rates. Following there is a brief summary detailing some key molecules involved in melanoma migration/invasion and metastasis; interested readers are suggested to read topic reviews elsewhere (Gaggioli and Sahai, 2007; Uong and Zon, 2010).

MITF plays a pivotal role in melanocyte and melanoma biology, as it is involved not only in the control of migration and invasion, but also in proliferation, survival, and differentiation of melanocytic cells (Levy *et al.*, 2006). The fine tuning of MITF activity enables melanoma cells to switch between a proliferative (high MITF) or invasive (low MITF) state, while very high levels promote differentiation and complete absence of MITF is incompatible with survival (Carreira *et al.*, 2006; Gray-Schopfer *et al.*, 2007).

Additionally, a number of growth factors and cytokines are upregulated as melanoma becomes more invasive, such as hepatocyte growth factor (HGF), TGF-β, IL-8, Nodal and several members of fibroblast growth factor (FGF) family (al-Alousi *et al.*, 1996; Albino *et al.*, 1991; Topczewska *et al.*, 2006). In addition to the autocrine effect on melanoma cells themselves upregulating cell motility genes, these factors are thought to have also paracrine pro-invasive effects, since they can signal to other cell types of the microenvironment, such as fibroblasts, to produce more pro-invasive molecules, like tenascin C and HGF (De Wever *et al.*, 2004). On the other hand, HGF receptor c-Met has also been shown to have pro-invasive effects in melanocytes and melanoma cells (McGill *et al.*, 2006).

Melanoma inhibitory activity (MIA) protein is highly expressed in malignant melanomas but not in melanocytes (Bosserhoff, 2005). Several studies have shown that MIA enhances melanocyte and melanoma migration and invasion (Tatzel *et al.*, 2005), and additionally have suggested a central role for MIA in early melanoma development by regulating important melanoma-related pathways (Bosserhoff, 2005).

The Snail family transcription factors Snail and Slug have also increased activity in melanoma, since they downregulate the expression of keratinocyte-interacting surface molecules, such as E-cadherin and occluding, while upregulate N-cadherin, favoring interaction with stromal cells and not keratinocytes (Gaggioli and Sahai, 2007; Kajita *et al.*, 2004). Furthermore, Snail co-ordinately upregulates the expression of cell motility genes including matrix metalloproteinase (MMP)- 2, secreted protein acidic and rich in cysteine (SPARC), tissue inhibitor of metalloproteinases 1 (TIMP-1) and RhoA (Kuphal *et al.*, 2005).

Recently several studies have highlighted that melanoma cells also display a high plasticity regarding their cell motility. Melanoma cells can migrate and invade with different modes of movement that presumably allow them to adapt to varying microenvironments (Sahai and Marshall, 2003; Sanz-Moreno *et al.*, 2008). The main switches of these melanoma cell motility programs are Rho-GTPases family members Rac and Rho (Sanz-Moreno et al., 2008),

which are overexpressed in cancer (Sahai, 2005). Rac promotes a more elongated shape that requires matrix metalloproteinases in order to invade, while RhoA favors a rounded morphology and a movement less dependent on proteases (Sanz-Moreno *et al.*, 2008). On the other hand, MMPs are upregulated in invasive melanoma and they are thought to promote melanoma dissemination, probably through different mechanisms since different MMPs are thought to have pro- or anti-invasive effects (Overall and Lopez-Otin, 2002).

3.3 PEDF as a brake for melanoma progression

As previously mentioned, the antiangiogenic activity of PEDF prompted the study of its potential antitumor effects. We (Fernandez-Garcia *et al.*, 2007; Garcia *et al.*, 2004) and others (Abe et al., 2004; Doll et al., 2003; Ek et al., 2006b) described a complex mechanism underlying the potent inhibition of melanoma metastasis by PEDF. PEDF's antitumor activity in melanoma and other tumors is based on its dual action on the tumor microenvironment and on the tumor cells themselves (Fernandez-Garcia *et al.*, 2007). PEDF inhibits tumor angiogenesis by means of induction of apoptosis on endothelial cells and modulation of the angiogenic profile of melanoma cells, eventually destroying the main source of nutrients to the primary tumor as well as one of the main routes of dissemination to distant organs. Additionally, PEDF exerts a potent inhibitory action on melanoma cells, inducing apoptosis under stress conditions (such as absence of growth factors or detachment from the extracellular matrix) and abrogating migration and invasion. As a whole, PEDF overexpression in melanoma cells leads to a decrease in primary tumor growth and an inhibition of lung metastasis formation (Fernandez-Garcia *et al.*, 2007; Filleur *et al.*, 2009).

In the previous section we described that PEDF is produced by low aggressive melanomas. Given that cutaneous melanoma develops from skin melanocytes, two questions soon arise: i) are normal melanocytes expressing this factor endogenously?, ii) if so, which could be the role of PEDF in normal melanocytic cells? By means of assessing a large collection of primary cultures of cutaneous melanocytes and other cell types of the skin we found that melanocytes secrete very high levels of PEDF comparable to other cell types known to express this factor (Orgaz *et al.*, 2009). In agreement with previous reports, dermal fibroblasts also express high levels of PEDF, which has been described to be involved in the control of their proliferative potential (Francis *et al.*, 2004; Tresini *et al.*, 1999); additionally, PEDF secreted by fibroblasts could also play a role in maintaining a correct vascularization of the skin. In contrast, PEDF is expressed at very low levels by epidermal keratinocytes or microvascular endothelial cells (Orgaz *et al.*, 2009).

In melanocytes, PEDF is one of the players involved in the regulation of their proliferation and migratory ability (Fig. 1). PEDF silencing in primary melanocytes leads to an increase in their migration and invasion, as well as a moderate augment in their growth rate (Orgaz *et al.*, 2009). Melanocytes arise from highly migratory embryonic neural crest progenitors, and therefore display an enormous migratory potential (Gupta *et al.*, 2005; Zbytek *et al.*, 2008) that must be tightly controlled in the skin (Hsu *et al.*, 2002). In addition to the regulatory signals from adjacent keratinocytes, an additional brake to their uncontrolled dissemination could be self-imposed within melanocytes by expressing high levels of PEDF themselves. As suggested for the fibroblasts, melanocytes could be also participating in the maintenance of appropriate angiogenesis in the skin by secreting this potent antiangiogenic factor, taking into account previous studies reporting an excessive vascularization of multiple organs upon PEDF knockdown (Doll *et al.*, 2003). Additional paracrine actions on other cell types, keratinocytes for instance, remain to be investigated.

Importantly, recent evidence points to a possible role of PEDF in melanocytic lineage-specific functions, such as pigment production. Melanocytes utilize specialized membrane vesicles called melanosomes to synthesize, store and deliver melanin to the cell membrane, eventually being transferred to surrounding keratinocytes (Barral and Seabra, 2004; Lin and Fisher, 2007). In a recent study we have found that PEDF overexpression in melanoma cell lines modulates the expression of Rab27A and melanophilin, two regulators of melanosome trafficking and melanin transfer (Orgaz *et al.*, 2011). Accordingly, previous reports had proposed the involvement of PEDF in pigment production, as PEDF was found in immature melanosomes of melanoma cells (Chi *et al.*, 2006) and it was also shown to induce tyrosinase expression (Abul-Hassan *et al.*, 2000) and melanosome maturation in RPE cells (Malchiodi-Albedi *et al.*, 1998). However, further studies are warranted in order to elucidate the functional role of PEDF in melanin production and/or secretion.

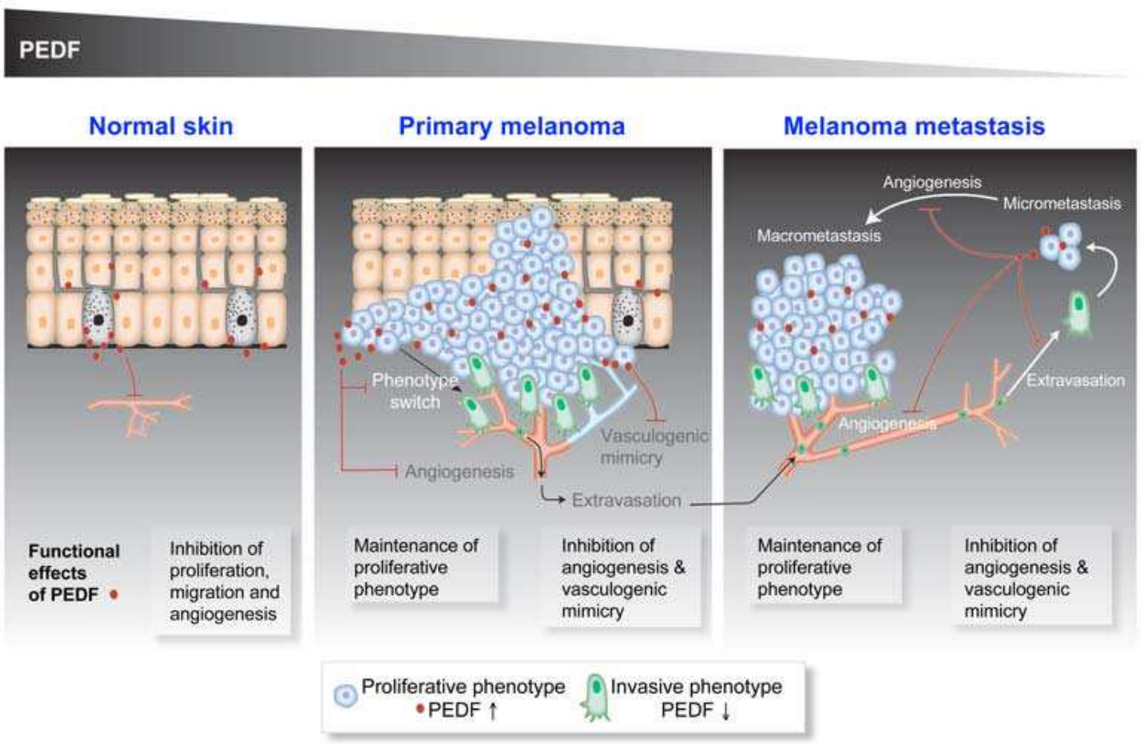


Fig. 1. Regulation of expression of PEDF during melanoma progression and functional effects in melanocytes and melanoma cells. Skin melanocytes express high levels of PEDF, which contribute to restrict their migratory and proliferative ability, and to regulate skin vascularization. In melanoma, PEDF expression is regulated by yet unidentified mechanisms that determine high levels in proliferative phenotype cells, and low levels in invasive phenotype cells. Melanoma progression is driven by switching between proliferative and invasive phenotypes, which involves reprogramming of PEDF and heterogeneous expression of this factor in primary and metastatic melanomas

The high expression of PEDF in skin melanocytes and its antitumor effects in melanoma raise the question whether PEDF expression could be modulated during melanoma development as it takes place in other types of cancer. However, defining melanoma

progression is a complex task. Therefore, we utilized different approaches in order to interrogate levels of PEDF during melanoma malignization (Orgaz *et al.*, 2009). When taking into account histopathological criteria (classical model of melanoma progression), PEDF expression is greatly diminished or lost in metastasis-derived melanoma cell lines compared to cell lines established from RGP or VGP tumors, even though we found a significant variability in PEDF levels across melanoma cell lines. Accordingly, PEDF expression is lower in metastatic melanoma biopsies compared to primary melanomas (Orgaz *et al.*, 2009; and unpublished data) (Fig. 1).

This first analysis was expanded taking advantage of the vast collection of publicly available gene expression data from large series of melanoma cell lines and primary cultures of melanocytes. Firstly, we confirmed the downregulation of PEDF expression in melanoma compared to primary melanocytes (Fig. 1) (Hoek, 2007; Orgaz *et al.*, 2009). When classifying a number of series of melanoma cell lines regarding their gene expression profile into proliferative or invasive phenotype, we found that there is a significant decrease of PEDF expression in invasive phenotype melanoma cell lines. This suggests again an inverse correlation between melanoma aggressiveness and invasiveness, and expression levels of PEDF (Orgaz *et al.*, 2009).

Furthermore, expression of PEDF is subject to certain plasticity and can be reprogrammed during the metastatic progression of melanoma. We utilized paired cell lines isolated from the same metastasis of a cutaneous melanoma patient, and that display extreme phenotypes, poorly or highly aggressive (Seftor *et al.*, 2005). Only the poorly aggressive melanoma cell line expresses PEDF at high levels comparable to those of melanocytes, while it is undetectable in the highly aggressive cell line. The heterogeneity of PEDF expression is also found *in vivo* when analyzed in biopsies from dermal or lymph node melanoma metastases. Most of the biopsies from metastases are negative for PEDF, but when positive they display a heterogeneous staining pattern of PEDF expression (Orgaz *et al.*, 2009). Both observations strongly suggest that PEDF expression could be reprogrammed during the metastatic process in melanoma, in the context of the phenotype switching model, being expressed only by the poorly aggressive subpopulation of tumor cells (Fig. 1). Regulatory signals from the tumor microenvironment could be responsible, at least in part, for this switching in PEDF expression. As an example, primary melanocytes grown on collagen matrix preconditioned by a highly aggressive melanoma cell line display decreased PEDF expression levels compared to melanocytes grown on untreated matrix (Seftor *et al.*, 2005).

The inverse correlation between melanoma aggressiveness and PEDF expression raises additional important questions. Firstly, what is the functional significance of PEDF modulation during melanoma progression? We addressed this by silencing endogenous PEDF expression in several melanoma cell lines utilizing short hairpin RNAs specific to PEDF delivered by lentiviral transduction. As in melanocytes, endogenous PEDF restricts the migratory and invasive ability of melanoma cells, which greatly translates *in vivo*: we found that PEDF inhibits spontaneous lung colonization by melanoma cells as well as formation of spontaneous lung metastases from a primary tumor (Orgaz *et al.*, 2009). Inhibition of spontaneous metastases formation by PEDF is particularly noteworthy, since it highlights that this factor also restricts the initial steps of the metastatic cascade, local invasiveness from the primary tumor and intravasation. Additionally, PEDF impinges on vasculogenic mimicry ability of melanoma cells (Fig. 1), diminishing the formation of vasculogenic networks by melanoma cells both *in vitro* on collagen matrices and *in vivo* in the lung parenchyma after tail vein injection into immunocompromised mice (Orgaz *et al.*, 2009).

Therefore, in the model that we propose reprogramming of PEDF expression is important for the switching between proliferative and invasive phenotypes that are thought to drive metastatic progression of melanoma. Unknown mechanisms so far determine that PEDF expression is high in proliferative phenotype primary tumor cells, where it restricts migratory and invasive abilities, angiogenic potential and vasculogenic mimicry, as a whole leading to a diminished metastatic potential. However, loss of PEDF helps melanoma cells to acquire an invasive phenotype essential to disseminate and colonize distant organs. There, some metastatic cells would be reprogrammed by signals from the new microenvironment toward proliferative phenotype cells, switching back to express PEDF again. Eventually these cells will develop micrometastases and macrometastases, where additional reprogramming events will take place leading to a metastatic lesion heterogeneous for PEDF expression. Additionally, mechanisms yet to be described determine a loss of PEDF expression in fully developed melanoma metastases (Fig. 1).

Despite the knowledge we have gathered regarding PEDF modulation in melanoma, important questions still remain to be resolved. First of all, which are the regulators of PEDF during melanoma progression? Elucidating the mechanisms that promote or repress PEDF expression would help better understand how PEDF changes in the course of transformation and malignant progression of melanoma. Our results suggest that PEDF expression could be modulated by two general types of mechanisms, reprogramming events and loss of expression.

Assessing PEDF expression during maturation and migration of neural crest precursors toward epidermis could allow investigating the relationship between migratory potential and differentiation state and PEDF expression in a physiological context. Eventually this could also help identify possible factors involved in reprogramming PEDF in the different environments that neural crest precursors encounter in their way toward epidermis. Some of these factors are likely to be responsible for modulating PEDF expression in melanoma cells, given that tumor cells frequently activate signaling pathways and utilize regulatory mechanisms typical of progenitor cells.

Signals from the microenvironment such as hypoxia and inflammation are thought to reprogram and switch melanoma cells toward an invasive phenotype, and therefore, could be responsible for the downregulation of PEDF during melanoma progression. As a matter of a fact, earlier reports described that PEDF expression is decreased by hypoxia in retinoblastoma (Dawson *et al.*, 1999) and RPE (Notari *et al.*, 2005). Additionally, transcription factors that drive and are at the core of each phenotype gene signature could be direct regulators of PEDF expression. MITF is an appealing candidate, since it is a key factor in melanoma and melanocyte biology (Levy *et al.*, 2006) with anti-invasive effects and whose expression is tightly associated with the proliferative phenotype (Carreira *et al.*, 2006; Hoek *et al.*, 2008). Similar to PEDF, some studies have reported a trend toward loss of MITF expression in metastases compared to primary melanomas (Carreira *et al.*, 2006; Goodall *et al.*, 2008).

Additionally, genetic and epigenetic mechanisms could lead to a loss of PEDF expression as melanoma evolves to metastatic. The expression of a number of angiogenesis regulators in cancer is controlled by gain of expression of oncogenes and loss of tumor suppressors (Bouck, 1990). PEDF is a direct target of p53-related p63 and p73 proteins in colorectal carcinoma (Sasaki *et al.*, 2005). In melanoma, TA-p73 isoform has been recently described to inhibit anchor independent growth through KCNK1 protein, whose expression decreases in melanoma compared to normal skin (Beitzinger *et al.*, 2008). Therefore it would be

interesting to assess whether p73 is implicated in regulating PEDF expression during melanoma progression. Human melanomas display either oncogenic NRAS (20% melanomas) or BRAF (50-70%), thought to be responsible of the initial transformation of melanocytes, uncoupling cell growth from external mitogenic stimuli (Chin, 2003; Chin *et al.*, 2006). However, by means of an analysis of publicly available microarray data from melanoma cell lines, we did not observe a significant correlation between PEDF expression levels and BRAF or NRAS mutation status (unpublished data). Finally, loss of expression of PEDF could occur upon epigenetic silencing of its promoter. Maspin, another member of Serpin family with anti-invasive and anti-tumor activities, is expressed at high levels in melanocytes but it is silenced in naevi and melanomas by epigenetic mechanisms (Denk *et al.*, 2007).

Although molecular aspects of the mechanism of action of PEDF on endothelial cells or neural derived cells have been described little is known about the molecular mechanisms underlying PEDF´s actions in melanoma, particularly in the inhibition of invasion and metastasis.

Interestingly, a recent study by us utilizing a gene expression analysis upon PEDF overexpression in melanoma cell lines has started to reveal some factors and pathways that could be mediating PEDF antimetastatic effects (Orgaz *et al.*, 2011). PEDF downregulates several key promigratory and proangiogenic factors such as IL-8, TGF-α and TGF-β, as well as a number of proteases and extracellular matrix proteins, like collagen IV, that could account for the lesser invasive ability and angiogenic potential of melanoma cells expressing PEDF. Additionally, PEDF modulates genes previously involved in melanoma progression toward a trend in agreement with decreased aggressiveness and invasiveness, such as factors from the Notch (Pinnix and Herlyn, 2007) or Wnt (Weeraratna, 2005) pathways, as well as FGF13 (Hoek *et al.*, 2004), insuling-like growth factor binding protein 3 (IGFBP3) (Xi *et al.*, 2006) or inhibin beta A (INHBA) (Hoek *et al.*, 2006), among many others. Interestingly, a number of melanoma markers with increased levels upon melanoma malignization are predominantly downregulated by PEDF overexpression, such as MIA or S100-β (Deichmann *et al.*, 1999; Utikal *et al.*, 2007).

4. Therapeutic applications of pigment epithelium derived-factor

4.1 Biochemical features of PEDF

Therapeutic applications of PEDF are closely related to the cellular niches where this factor is produced and to the multiplicity of cellular functions and activities ascribed to this secreted serpin family member.

SERPINs are a large superfamily of genes that codes for serine protease inhibitors in mammals (Tombran-Tink *et al.*, 2005). These proteins are able to control several processes such as blood coagulation, complement activation and extracellular matrix remodelling (Filleur *et al.*, 2009; Tombran-Tink *et al.*, 2005). However, there is a small number of serpin family members with non-inhibitory protease activity, among which is included PEDF (*SERPINF1*) (Becerra *et al.*, 1995; Lawrence *et al.*, 1990; Steele *et al.*, 1993). The inhibitory activity against proteinases resides in a domain called reactive centre loop (RCL). The reason why PEDF lacks protease inhibitory capability is due to the presence in the RCL of several proline residues preceding the cleavage site (Simonovic *et al.*, 2001).

Amino acid analyses indicate that PEDF shares considerable sequence homology with other members of the serpin family (Steele *et al.*, 1993); however, residues 40-67, at the N-terminal

(N-ter) region, and residues 277-301 at C-terminal (C-ter) are specific to PEDF. This feature suggests that these residues could be involved in maintaining the distinct structure of PEDF in these regions or in determining specific functions of this non-inhibitory serpin (Tombran-Tink *et al.*, 2005; Xu *et al.*, 2006). Tombran-Tink and collaborators also compared sequence homology of PEDF protein among different species and analyzed structural homology. They found a high degree of conservation in the leader sequence, responsible for protein secretion, a C-ter glycosilation site, and four specific regions: two domains present at N-ter region, corresponding to residues 40-67 and 78-95; and other two regions, 277-301 and 384-415, located at C-ter of the protein (Tombran-Tink *et al.*, 2005).

PEDF was initially isolated from conditioned medium of human fetal RPE cells and identified as a neurotrophic factor (Tombran-Tink and Johnson, 1989), although further studies showed that it is widely expressed throughout fetal and adult tissues (Ek *et al.*, 2006b). This broad expression is suggestive of a general and relevant function of PEDF in mammals. PEDF is also known as EPC-1 (early population doubling cDNA-1), and has been shown to participate in cell cycle regulation, initially in fibroblasts (Pignolo *et al.*, 1993; Tombran-Tink and Johnson, 1989; Tombran-Tink *et al.*, 1995) and later in other cell types like endothelial cells (Duh *et al.*, 2002; Hutchings *et al.*, 2002). PEDF's cell cycle regulatory function requires the presence of a putative nuclear localization sequence (residues 141-151) that is highly conserved in *SERPINF1* among different species, and could mediate PEDF translocation to the nucleus (Tombran-Tink *et al.*, 2005). One of the most relevant functional characteristics of PEDF is its antiangiogenic activity, being considered as the most potent natural inhibitor of physiological and pathological angiogenesis (Dawson *et al.*, 1999). PEDF is also a very effective neurotrophic factor that induces cell differentiation, cell survival, and protection from cell death in many cell types of the nervous system. PEDF prevents degeneration of retinal neurons that are exposed to transient ischemic reperfusion (Ogata *et al.*, 2001), and also protects other regions of the brain and spinal cord from the damaging effects caused by oxidative stress and glutamate toxicity (Bilak *et al.*, 1999; Cao *et al.*, 1999; DeCoster *et al.*, 1999; Taniwaki *et al.*, 1995). It has been shown the implication of NFkB in all these processes, which induces the expression of neurotrophic factors and anti-apoptotic genes that participate in the control of cell survival, proliferation and death (Barnstable and Tombran-Tink, 2004; Yabe *et al.*, 2001). Neurotrophic activity has been mapped to the N-ter region of PEDF, encompassing residues Val^{58}-Thr^{101} (Simonovic *et al.*, 2001). This region is involved in binding to a putative plasma membrane receptor in cerebellar granule neurons and retinoblastoma cells (Alberdi *et al.*, 1999). PEDF is also secreted by ependymal and endothelial cells from the subventricular zone of the brain, where it promotes the self-renewal of adult neural stem cells (Andreu-Agullo *et al.*, 2009; Ramirez-Castillejo *et al.*, 2006). Furthermore, PEDF presents three phosphorylation sites located in Ser^{24}, Ser^{114} and Ser^{227}, and the regulation of their phosphorylation state modulates the switch of PEDF's function from neurotrophic (Ser^{24} and Ser^{114} phosphorylated) to angiangiogenic (Ser^{227} phosphorylated) (Becerra, 2006).

In order to better understand the mechanism of action of PEDF, it was necessary to identify the structural domains responsible for each of its described biological functions. Two major epitopes were identified at the N-ter region of PEDF: 34-mer peptide (residues 24-57) responsible of the antiangiogenic and pro-apoptotic actions of PEDF; and a second epitope, 44-mer peptide (residues 58-101), which induces neuronal differentiation of retinoblastoma cells (Filleur *et al.*, 2005) and plays a neurotrophic role in many neuronal cell types (Bilak *et al.*, 2002). There are another two highly conserved smaller epitopes, one upstream 34-mer,

the TGA epitope, and an internal fragment of 44-mer, referred to as ERT. Filleur and collaborators showed *in vivo* that the epitopes TGA and the complete 34-mer inhibit tumor angiogenesis in prostate adenocarcinoma by inducing apoptosis of endothelial cells and blocking endothelial cell migration. Curiously, ERT despite of being located inside the neurotrophic 44-mer peptide, also presents antiangiogenic activity in prostate adenocarcinoma. Complete 44-mer is able to induce neurite outgrowth in Y-79 retinoblastoma cell line and causes apoptosis of endothelial cells, blocking migration and angiogenesis (Filleur *et al.*, 2005). In a more recent study, smaller regions of 34-mer epitope (named P14, P18 and P23, according to their respective length) were tested for angioinhibitory activity *in vitro* and *in vivo*. P14 and P23 display antiangiogenic activity *in vitro* (both blocking endothelial chemotaxis, and inducing apoptosis in the case of P23), but not *in vivo*; while P18 is a more potent antiangiogenic peptide than 34-mer in prostate cancer, being able to block bFGF and VEGF-dependent angiogenesis in the *in vivo* cornea neovascularisation assay (Mirochnik *et al.*, 2009). Another study by Ek and colleagues identified other small peptides with antitumoral activity in an orthotopic osteosarcoma model. They generated six 25-mer peptides along the functionally distinct regions of PEDF characterized so far. Residues 78-102 inhibit proliferation, whereas residues 90-114 stimulate adhesion of PEDF to type I collagen. Furthermore, residues 387 to 411 inhibit invasion of osteosarcoma cells *in vitro* and residues 40-64 promote osteogenic differentiation (Ek *et al.*, 2007).

The multifunctional character of PEDF and the evidence of the different roles displayed depending on the cell type, suggest that PEDF could be acting through distinct domains recognized by several specific receptors. The identification of these putative receptors and the characterization of the binding affinity of each functionally identified peptide toward them could make a breakthrough in the understanding of PEDF's mechanism of action and, therefore, the possibility of its therapeutic use in multiple pathological contexts.

4.2 PEDF receptors

Several studies have characterized the affinity of PEDF for the surface of human retinoblastoma cells, cerebellar granular neurons (Alberdi *et al.*, 1999), motor neurons (Bilak *et al.*, 2002), neural retina (Aymerich *et al.*, 2001) and endothelial cells (Yamagishi *et al.*, 2004). PEDF could be sequestered in the extracellular matrix based on ionic interactions with sulphated (heparin, heparin sulfate and condroitin sulfate) (Alberdi *et al.*, 1998), and non-sulfated (hyaluronan) (Becerra *et al.*, 2008) glycosaminglycans and type I collagen (Meyer *et al.*, 2002). In order to identify the potential receptor(s) of PEDF, Simonovic and collaborators carried out a three-dimensional study of PEDF, and identified an asymmetric charge distribution, with a high acidic region located at C-ter and basic amino acids at opposite region of PEDF protein (Simonovic *et al.*, 2001). This basic region is involved in the binding of PEDF to heparin through three clustered basic amino acid residues, Lys^{146}, Lys^{147} and Arg^{149}. Moreover, Asp^{256}, Asp^{258} and Asp^{300} residues present in the acidic region of PEDF are crucial to type I collagen binding (Meyer *et al.*, 2002; Yasui *et al.*, 2003).

Using the 34-mer and 44-mer epitopes it was possible to propose the existence of two distinct putative receptors for PEDF. These epitopes are able to bind the surface of endothelial and prostate cells, but they do not compete for receptor binding (Filleur *et al.*, 2005). This result suggests the existence of two PEDF receptors with different functions: PEDF-R^N, that interacts with 44-mer epitope and regulates the neurotrophic and neuroprotective activities of PEDF; and PEDF-R^A, which is involved in blocking

angiogenesis by binding to 34-mer epitope (Fig. 2). The differential expression of these two putative PEDF receptors in the diverse cell types analyzed supports the idea of distinct functions for each receptor type. PEDF-R^N is an 80-kDa receptor, which is located on the surface of human retinoblastoma cells, neural retina, cerebellar granular and motor neurons, whereas PEDF-R^A is a 60-kDa receptor specifically present on endothelial cells (Filleur *et al.*, 2009).

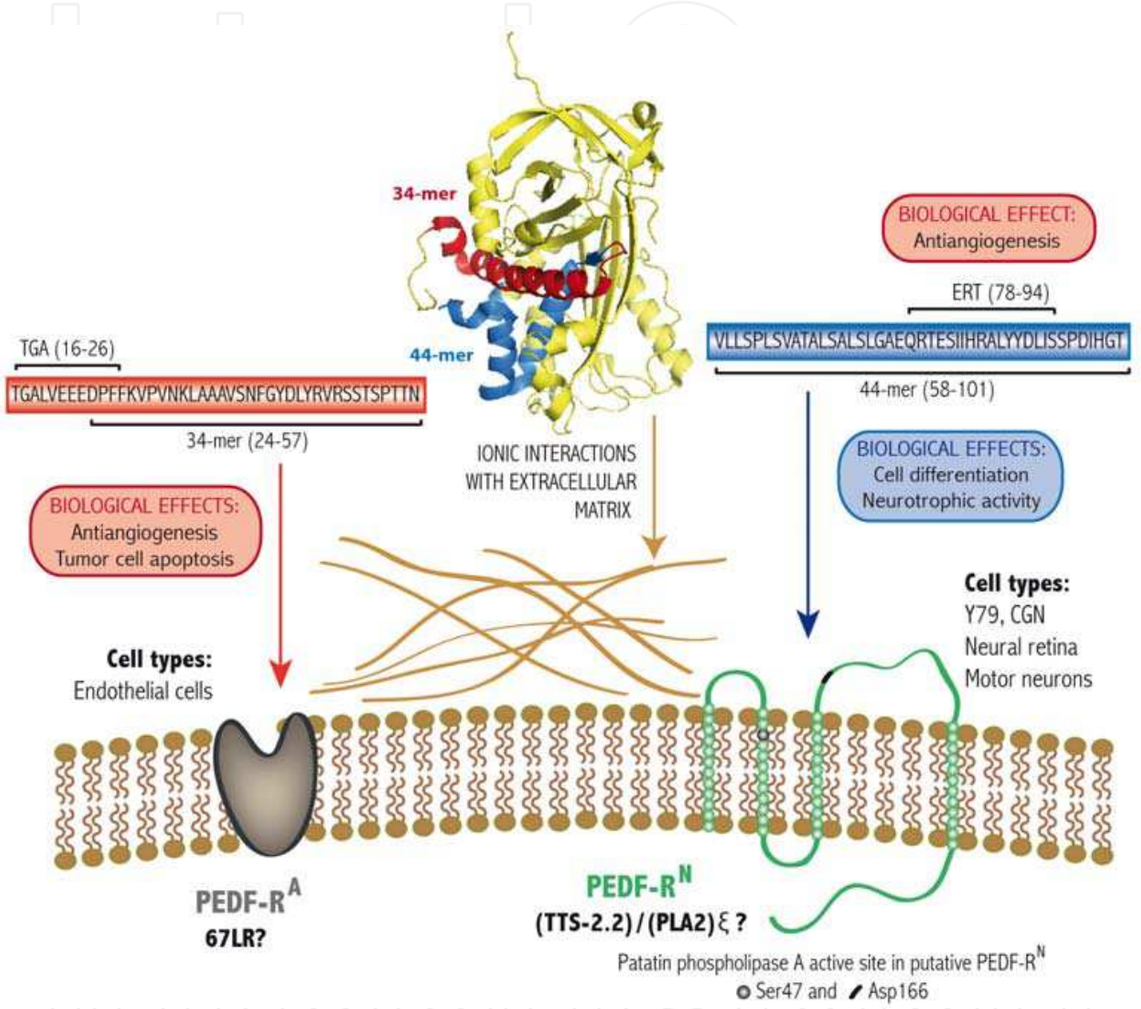


Fig. 2. Three-dimensional structure of PEDF, small peptides derived from PEDF and receptors. Crystal structure of PEDF molecule showing the location of 34-mer and 44-mer peptides. PEDF could be sequestered in the extracellular matrix by ionic interactions with type I collagen and glycosaminglycans. 34-mer, TGA and ERT peptides display antiangiogenic action through their bind to 60-kDa receptor (PEDF-R^A, proposed as the non-integrin 67-kDa laminin receptor) in endothelial cells. 34-mer also has the capability to induce apoptosis in tumor cells. 44-mer and ERT peptides present a neurotrophic action through their binding to the TTS-2.2/ (PLA2) ξ receptor (putatively identified as the PEDF-R^N) in several cell types. Abbreviations: 67LR: Non-Integrin 67-kDa laminin receptor; TTS-2.2/(PLA2)ξ: Transport secretion protein 2.2/Independent phospholipase A_2; Y79: human retinoblastoma cell line; CGN: cerebellar granular neurons

Further studies identified on the surface of the retina and human immortalized retinoblastoma cells (ARPE-19) the human Transport Secretion Protein 2-2 (TTS-2.2) /Independent phospholipase A_2 (PLA_2)ξ (also known in mice as adipose triglyceride lipase-ATGL, desnutrin, and patatin-like phospholipase domain containing protein-PNPLP2), an important lipase involved in triglyceride metabolism, as a specific PEDF receptor (Notari *et al.*, 2006). The phospholipase A_2 domain of this receptor releases bioactive fatty acids that function as second messengers. Therefore, depending on the lipid released, this receptor could activate different signal transduction pathways. It is still not fully demonstrated if the 80-kDa (PLA2)ξ receptor is the previously identified PEDF-R^N receptor (Fig. 2).
Recently, a new receptor for PEDF has been identified, the non-integrin 67-kDa laminin receptor (67LR) (Bernard *et al.*, 2009), which may be related to the 60-kDa receptor previously reported in endothelial cells (Yamagishi *et al.*, 2004). This hypothesis is supported by the observation of antiangiogenic effects (inhibition of endothelial cell migration and induction of endothelial cell apoptosis) when 34-mer epitope binds to 67LR and the previously described 60-kDa receptor.
However, whether these characterized receptors are expressed on the surface of melanocytes and melanoma cells to mediate multiple PEDF's biological activities, and whether other unknown melanocytic lineage-specific receptors could play a role in translating PEDF's effects, remains to be investigated.

4.3 Therapeutic prospects for PEDF

During the last two decades many groups have described PEDF as a multifunctional protein that plays effective neuroprotective and antiangiogenic activities. The wide diversity of PEDF functions, along with the fact that it is an endogenous molecule, makes PEDF a unique candidate for a therapeutic agent in many diseases.
Several studies have already shown the role of PEDF in various pathological conditions such as ocular and chronic inflammatory diseases, atherosclerosis and diabetes. Moreover, several groups have demonstrated, *in vivo* and *in vitro*, the antitumor and antimetastatic potential of PEDF. They described the capability of PEDF to induce tumor cell differentiation in neuroblastoma (Crawford *et al.*, 2001) and prostate cancer (Filleur *et al.*, 2005); a direct tumor suppression action in osteosarcoma (Takenaka *et al.*, 2005), melanoma (Abe *et al.*, 2004; Garcia *et al.*, 2004) and prostate cancer (Filleur *et al.*, 2005; Guan *et al.*, 2007); and its angiostatic action in a wide number of tumor types that include, among others, melanoma (Abe *et al.*, 2004; Garcia *et al.*, 2004; Orgaz *et al.*, 2009; Yang and Grossniklaus, 2010).
The use of PEDF as a therapeutic agent in the clinic requires deeper knowledge of its biological effects and underlying molecular mediators. An important feature is the development of effective therapeutic agents based on the optimum combination of biological activities beneficial for each specific pathological context, together with the development of optimum delivery systems to allow proper targeting and stability during treatment. One of the possibilities is the use of recombinant full-length PEDF protein (rPEDF) produced in human embryonic kidney cells. Also, as PEDF is secreted at high levels by RPE cells, endogenous PEDF protein can be purified from the conditioned medium of RPEs. rPEDF has been successfully tested *in vitro* and *in vivo* in osteosarcoma (Takenaka *et al.*, 2005), prostate cancer (Doll *et al.*, 2003) and neuroblastoma (Crawford *et al.*, 2001). Due to its endogenous nature, short-term treatment would not lead to any immune response after systemic administration. However, the main disadvantage of this strategy is the susceptibility of rPEDF to cleavage by proteases, and therefore a limited bioavailability.

PEDF is a protein that is 418 amino acids in length. Filleur and collaborators elucidated that two small fragments of PEDF, 34-mer and 44-mer, were able to display the antiangiogenic and neurotrophic roles (respectively) as the complete protein (Filleur *et al*., 2005). Shorter peptides improve the stability and delivery, and reduce the possibility of being recognized by the immune system. However, the use of rPEDF or small peptides derived from functionally active regions requires a systemic distribution; and therefore due to the antiangiogenic activity of this factor or derived peptides, its presence in plasma could have unexplored side effects on physiologic vascularization during the menstrual cycle and wound healing (Ek *et al*., 2006a).

An alternative strategy that has been widely tested by a number of groups in mouse models of cancer is the use of viral vectors for the delivery of either full length PEDF or small peptides derived from the diverse functional regions identified. Gene transfer using viral or plasmid vectors is an attractive tool for human cancer gene therapy. Several studies have used this strategy in different types of cancer, such as pancreatic cancer (Hase *et al*., 2005), neuroblastoma (Streck *et al*., 2005), prostate cancer (Guan *et al*., 2007) and melanoma (Abe *et al*., 2004; Garcia *et al*., 2004; Orgaz *et al*., 2009; Yang and Grossniklaus, 2010), with a reduction of primary tumor size and number of metastases after PEDF delivery.

In melanoma, Abe's group and us were the first to describe the antitumor effect of PEDF *in vitro* and *in vivo* in malignant melanoma cell lines (Abe *et al*., 2004; Garcia *et al*., 2004). To this aim, Abe and collaborators overexpressed PEDF by transfection of G361 melanoma cell line and observed in nude mice a reduction of tumor angiogenesis and an almost complete inhibition of G361 growth in melanoma xenografts. These effects are the result of suppression of tumor angiogenesis and induction of Fas ligand-dependent apoptosis in tumor cells (Abe *et al*., 2004). We used a retroviral strategy to transduce the human melanoma cell line UCD-Mel-N, which does not express significant levels of endogenous PEDF. We observed a considerable inhibition of primary tumor growth in subcutaneous xenotransplants in immunocompromised mice and a complete abrogation of lung metastases formation in the tail vein injection model. We demonstrated that the inhibition of primary melanoma tumor growth by PEDF is based on selective destruction of immature vessels, together with a significant direct induction of apoptosis in melanoma cells. Although it was first demonstrated that PEDF inhibits endothelial cell migration and induces apoptosis in remodelling endothelium, we showed that PEDF also has direct effects on melanoma cells, inhibiting melanoma cell migration and inducing apoptosis under stress conditions like absence of growth factors or detachment from the extracellular matrix (Garcia *et al*., 2004). In a recent work we also used a lentiviral transduction strategy to silence PEDF in poorly aggressive melanoma cell lines with high expression of endogenous PEDF. PEDF knockdown in these melanoma cell lines enables the acquisition of an invasive phenotype, showing the critical importance of PEDF for the malignant progression of human melanoma (Orgaz *et al*., 2009).

Retroviral and lentiviral vectors are attractive tools for human cancer gene therapy and, based on their ability to integrate into the genome, they have the potential to achieve long-term stable expression and maintain therapeutic levels of secreted peptides (Hase *et al*., 2005). Both types of virus are able to transduce proliferating cells, although only lentiviruses present the advantage of transducing non-dividing cells. This feature is of great advantage for gene transfer as a complementary treatment in cancer, due to the fact that chemotherapy is only effective in actively proliferative cells, allowing non-dividing cells to be resistant to treatment and enabling the development of metastasis. Although viral systems seem to be a

promising therapy for cancer and other diseases, there are still some problems and patient risks that have to be solved, such as (i) obtaining clinically effective viral titres, (ii) stable transgene expression in individuals requiring long-term treatment, and (iii) the risk of *de novo* cancer initiation via recombination within the patient's cell genome.

The biological effect of small peptides derived from PEDF retaining different functional activities has not yet been explored in melanoma, although it would be very relevant for the therapeutic development of PEDF in the context of aggressive melanoma. Also, characterization of PEDF's receptors expressed in melanoma cells is very important in order to understand the molecular mechanism of action underpinning the multiple biological actions of PEDF on melanoma cells, as well as to develop new therapeutic strategies based on the receptors and pathways that mediate PEDF's actions in aggressive melanoma.

5. Conclusions

Collectively, our findings strongly support PEDF as a potent biological modifier that effectively halts the metastatic spread of human melanoma by combining distinct functional epitopes respectively impinging on the vascular component of the tumor microenvironment and on the set of capabilities that a melanoma cell must acquire in order to successfully leave its primary site to colonize distant foreign environments.

6. Acknowledgment

Supported by grants Ministerio de Educacion y Ciencia SAF2007-62292 and SAF2010-19256 to BJ. AFB has been supported by a CSIC-JAE fellowship and JLO by a SAF2007-62292 contract.

7. References

Abe R., Shimizu T., Yamagishi S., Shibaki A., Amano S., Inagaki Y., Watanabe H., Sugawara H., Nakamura H., Takeuchi M., Imaizumi T., & Shimizu H. (2004). Overexpression of pigment epithelium-derived factor decreases angiogenesis and inhibits the growth of human malignant melanoma cells in vivo. *Am J Pathol* 164: 1225-32.

Abramson LP., Stellmach V., Doll JA., Cornwell M., Arensman RM., & Crawford SE. (2003). Wilms' tumor growth is suppressed by antiangiogenic pigment epithelium-derived factor in a xenograft model. *J Pediatr Surg* 38: 336-42; discussion 336-42.

Abul-Hassan K., Walmsley R., Tombran-Tink J., & Boulton M. (2000). Regulation of tyrosinase expression and activity in cultured human retinal pigment epithelial cells. *Pigment Cell Res* 13: 436-41.

al-Alousi S., Carlson JA., Blessing K., Cook M., Karaoli T., & Barnhill RL. (1996). Expression of basic fibroblast growth factor in desmoplastic melanoma. *J Cutan Pathol* 23: 118-25.

Alberdi E., Hyde CC., & Becerra SP. (1998). Pigment epithelium-derived factor (PEDF) binds to glycosaminoglycans: analysis of the binding site. *Biochemistry* 37: 10643-52.

Alberdi E., Aymerich MS., & Becerra SP. (1999). Binding of pigment epithelium-derived factor (PEDF) to retinoblastoma cells and cerebellar granule neurons. Evidence for a PEDF receptor. *J Biol Chem* 274: 31605-12.

Albino AP., Davis BM., & Nanus DM. (1991). Induction of growth factor RNA expression in human malignant melanoma: markers of transformation. *Cancer Res* 51: 4815-20.

Andreu-Agullo C., Morante-Redolat JM., Delgado AC., & Farinas I. (2009). Vascular niche factor PEDF modulates Notch-dependent stemness in the adult subependymal zone. *Nat Neurosci* 12: 1514-23.

Aurora AB., Biyashev D., Mirochnik Y., Zaichuk TA., Sanchez-Martinez C., Renault MA., Losordo D., & Volpert OV. (2010). NF-kappaB balances vascular regression and angiogenesis via chromatin remodeling and NFAT displacement. *Blood* 116: 475-84.

Aymerich MS., Alberdi EM., Martinez A., & Becerra SP. (2001). Evidence for pigment epithelium-derived factor receptors in the neural retina. *Invest Ophthalmol Vis Sci* 42: 3287-93.

Baluk P., Hashizume H., & McDonald DM. (2005). Cellular abnormalities of blood vessels as targets in cancer. *Curr Opin Genet Dev* 15: 102-11.

Barnstable CJ., & Tombran-Tink J. (2004). Neuroprotective and antiangiogenic actions of PEDF in the eye: molecular targets and therapeutic potential. *Prog Retin Eye Res* 23: 561-77.

Barral DC., & Seabra MC. (2004). The melanosome as a model to study organelle motility in mammals. *Pigment Cell Res* 17: 111-8.

Basu B., Biswas S., Wrigley J., Sirohi B., & Corrie P. (2009). Angiogenesis in cutaneous malignant melanoma and potential therapeutic strategies. *Expert Rev Anticancer Ther* 9: 1583-98.

Becerra SP., Sagasti A., Spinella P., & Notario V. (1995). Pigment epithelium-derived factor behaves like a noninhibitory serpin. Neurotrophic activity does not require the serpin reactive loop. *J Biol Chem* 270: 25992-9.

Becerra SP. (2006). Focus on Molecules: Pigment epithelium-derived factor (PEDF). *Exp Eye Res* 82: 739-40.

Becerra SP., Perez-Mediavilla LA., Weldon JE., Locatelli-Hoops S., Senanayake P., Notari L., Notario V., & Hollyfield JG. (2008). Pigment epithelium-derived factor binds to hyaluronan. Mapping of a hyaluronan binding site. *J Biol Chem* 283: 33310-20.

Bedogni B., & Powell MB. (2009). Hypoxia, melanocytes and melanoma - survival and tumor development in the permissive microenvironment of the skin. *Pigment Cell Melanoma Res* 22: 166-74.

Beitzinger M., Hofmann L., Oswald C., Beinoraviciute-Kellner R., Sauer M., Griesmann H., Bretz AC., Burek C., Rosenwald A., & Stiewe T. (2008). p73 poses a barrier to malignant transformation by limiting anchorage-independent growth. *Embo J* 27: 792-803.

Bergers G., & Hanahan D. (2008). Modes of resistance to anti-angiogenic therapy. *Nat Rev Cancer* 8: 592-603.

Bernard A., Gao-Li J., Franco CA., Bouceba T., Huet A., & Li Z. (2009). Laminin receptor involvement in the anti-angiogenic activity of pigment epithelium-derived factor. *J Biol Chem* 284: 10480-90.

Bilak MM., Corse AM., Bilak SR., Lehar M., Tombran-Tink J., & Kuncl RW. (1999). Pigment epithelium-derived factor (PEDF) protects motor neurons from chronic glutamate-mediated neurodegeneration. *J Neuropathol Exp Neurol* 58: 719-28.

Bilak MM., Becerra SP., Vincent AM., Moss BH., Aymerich MS., & Kuncl RW. (2002). Identification of the neuroprotective molecular region of pigment epithelium-derived factor and its binding sites on motor neurons. *J Neurosci* 22: 9378-86.

Bittner M., Meltzer P., Chen Y., Jiang Y., Seftor E., Hendrix M., Radmacher M., Simon R., Yakhini Z., Ben-Dor A., Sampas N., Dougherty E., Wang E., Marincola F., Gooden C., Lueders J., Glatfelter A., Pollock P., Carpten J., Gillanders E., Leja D., Dietrich K., Beaudry C., Berens M., Alberts D., & Sondak V. (2000). Molecular classification of cutaneous malignant melanoma by gene expression profiling. *Nature* 406: 536-40.

Bosserhoff AK. (2005). Melanoma inhibitory activity (MIA): an important molecule in melanoma development and progression. *Pigment Cell Res* 18: 411-6.

Bouck N. (1990). Tumor angiogenesis: the role of oncogenes and tumor suppressor genes. *Cancer Cells* 2: 179-85.

Bouck N. (2002). PEDF: anti-angiogenic guardian of ocular function. *Trends Mol Med* 8: 330-4.

Broadhead ML., Dass CR., & Choong PF. (2009). In vitro and in vivo biological activity of PEDF against a range of tumors. *Expert Opin Ther Targets* 13: 1429-38.

Cai J., Parr C., Watkins G., Jiang WG., & Boulton M. (2006). Decreased pigment epithelium-derived factor expression in human breast cancer progression. *Clin Cancer Res* 12: 3510-7.

Cao W., Tombran-Tink J., Chen W., Mrazek D., Elias R., & McGinnis JF. (1999). Pigment epithelium-derived factor protects cultured retinal neurons against hydrogen peroxide-induced cell death. *J Neurosci Res* 57: 789-800.

Carmeliet P. (2005). Angiogenesis in life, disease and medicine. *Nature* 438: 932-6.

Carreira S., Goodall J., Denat L., Rodriguez M., Nuciforo P., Hoek KS., Testori A., Larue L., & Goding CR. (2006). Mitf regulation of Dia1 controls melanoma proliferation and invasiveness. *Genes Dev* 20: 3426-39.

Chi A., Valencia JC., Hu ZZ., Watabe H., Yamaguchi H., Mangini NJ., Huang H., Canfield VA., Cheng KC., Yang F., Abe R., Yamagishi S., Shabanowitz J., Hearing VJ., Wu C., Appella E., & Hunt DF. (2006). Proteomic and bioinformatic characterization of the biogenesis and function of melanosomes. *J Proteome Res* 5: 3135-44.

Chin L. (2003). The genetics of malignant melanoma: lessons from mouse and man. *Nat Rev Cancer* 3: 559-70.

Chin L., Garraway LA., & Fisher DE. (2006). Malignant melanoma: genetics and therapeutics in the genomic era. *Genes Dev* 20: 2149-82.

Clark WH, Jr. (1991). Human cutaneous malignant melanoma as a model for cancer. *Cancer Metastasis Rev* 10: 83-88.

Crawford SE., Stellmach V., Ranalli M., Huang X., Huang L., Volpert O., De Vries GH., Abramson LP., & Bouck N. (2001). Pigment epithelium-derived factor (PEDF) in neuroblastoma: a multifunctional mediator of Schwann cell antitumor activity. *J Cell Sci* 114: 4421-8.

Dawson DW., Volpert OV., Gillis P., Crawford SE., Xu H., Benedict W., & Bouck NP. (1999). Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. *Science* 285: 245-8.

De Wever O., Nguyen QD., Van Hoorde L., Bracke M., Bruyneel E., Gespach C., & Mareel M. (2004). Tenascin-C and SF/HGF produced by myofibroblasts in vitro provide convergent pro-invasive signals to human colon cancer cells through RhoA and Rac. *FASEB J* 18: 1016-8.

DeCoster MA., Schabelman E., Tombran-Tink J., & Bazan NG. (1999). Neuroprotection by pigment epithelial-derived factor against glutamate toxicity in developing primary hippocampal neurons. *J Neurosci Res* 56: 604-10.

Deichmann M., Benner A., Bock M., Jackel A., Uhl K., Waldmann V., & Naher H. (1999). S100-Beta, melanoma-inhibiting activity, and lactate dehydrogenase discriminate progressive from nonprogressive American Joint Committee on Cancer stage IV melanoma. *J Clin Oncol* 17: 1891-6.

Denk AE., Bettstetter M., Wild PJ., Hoek K., Bataille F., Dietmaier W., & Bosserhoff AK. (2007). Loss of maspin expression contributes to a more invasive potential in malignant melanoma. *Pigment Cell Res* 20: 112-9.

Doll JA., Stellmach VM., Bouck NP., Bergh AR., Lee C., Abramson LP., Cornwell ML., Pins MR., Borensztajn J., & Crawford SE. (2003). Pigment epithelium-derived factor regulates the vasculature and mass of the prostate and pancreas. *Nat Med* 9: 774-80.

Duh EJ., Yang HS., Suzuma I., Miyagi M., Youngman E., Mori K., Katai M., Yan L., Suzuma K., West K., Davarya S., Tong P., Gehlbach P., Pearlman J., Crabb JW., Aiello LP., Campochiaro PA., & Zack DJ. (2002). Pigment epithelium-derived factor suppresses ischemia-induced retinal neovascularization and VEGF-induced migration and growth. *Invest Ophthalmol Vis Sci* 43: 821-9.

Ek ET., Dass CR., & Choong PF. (2006a). PEDF: a potential molecular therapeutic target with multiple anti-cancer activities. *Trends Mol Med* 12: 497-502.

Ek ET., Dass CR., & Choong PF. (2006b). Pigment epithelium-derived factor: a multimodal tumor inhibitor. *Mol Cancer Ther* 5: 1641-6.

Ek ET., Dass CR., Contreras KG., & Choong PF. (2007). PEDF-derived synthetic peptides exhibit antitumor activity in an orthotopic model of human osteosarcoma. *J Orthop Res* 25: 1671-80.

Ellis LM., & Hicklin DJ. (2008). VEGF-targeted therapy: mechanisms of anti-tumour activity. *Nat Rev Cancer* 8: 579-91.

Fecher LA., Cummings SD., Keefe MJ., & Alani RM. (2007). Toward a molecular classification of melanoma. *J Clin Oncol* 25: 1606-20.

Fernandez-Garcia NI., Volpert OV., & Jimenez B. (2007). Pigment epithelium-derived factor as a multifunctional antitumor factor. *J Mol Med* 85: 15-22.

Ferrara N., & Kerbel RS. (2005). Angiogenesis as a therapeutic target. *Nature* 438: 967-74.

Filleur S., Volz K., Nelius T., Mirochnik Y., Huang H., Zaichuk TA., Aymerich MS., Becerra SP., Yap R., Veliceasa D., Shroff EH., & Volpert OV. (2005). Two functional epitopes of pigment epithelial-derived factor block angiogenesis and induce differentiation in prostate cancer. *Cancer Res* 65: 5144-52.

Filleur S., Nelius T., de Riese W., & Kennedy RC. (2009). Characterization of PEDF: a multi-functional serpin family protein. *J Cell Biochem* 106: 769-75.

Folberg R., Hendrix MJ., & Maniotis AJ. (2000). Vasculogenic mimicry and tumor angiogenesis. *Am J Pathol* 156: 361-81.

Folkman J. (2006). Angiogenesis. *Annu Rev Med* 57: 1-18.

Francis MK., Appel S., Meyer C., Balin SJ., Balin AK., & Cristofalo VJ. (2004). Loss of EPC-1/PEDF expression during skin aging in vivo. *J Invest Dermatol* 122: 1096-105.

Gaggioli C., & Sahai E. (2007). Melanoma invasion - current knowledge and future directions. *Pigment Cell Res* 20: 161-72.

Gao D., Nolan DJ., Mellick AS., Bambino K., McDonnell K., & Mittal V. (2008). Endothelial progenitor cells control the angiogenic switch in mouse lung metastasis. *Science* 319: 195-8.

Garcia M., Fernandez-Garcia NI., Rivas V., Carretero M., Escamez MJ., Gonzalez-Martin A., Medrano EE., Volpert O., Jorcano JL., Jimenez B., Larcher F., & Del Rio M. (2004). Inhibition of xenografted human melanoma growth and prevention of metastasis development by dual antiangiogenic/antitumor activities of pigment epithelium-derived factor. *Cancer Res* 64: 5632-42.

Goodall J., Carreira S., Denat L., Kobi D., Davidson I., Nuciforo P., Sturm RA., Larue L., & Goding CR. (2008). Brn-2 represses microphthalmia-associated transcription factor expression and marks a distinct subpopulation of microphthalmia-associated transcription factor-negative melanoma cells. *Cancer Res* 68: 7788-94.

Goss PE., & Chambers AF. (2010). Does tumour dormancy offer a therapeutic target? *Nat Rev Cancer* 10: 871-7.

Gray-Schopfer V., Wellbrock C., & Marais R. (2007). Melanoma biology and new targeted therapy. *Nature* 445: 851-7.

Guan M., Yam HF., Su B., Chan KP., Pang CP., Liu WW., Zhang WZ., & Lu Y. (2003). Loss of pigment epithelium derived factor expression in glioma progression. *J Clin Pathol* 56: 277-82.

Guan M., Jiang H., Xu C., Xu R., Chen Z., & Lu Y. (2007). Adenovirus-mediated PEDF expression inhibits prostate cancer cell growth and results in augmented expression of PAI-2. *Cancer Biol Ther* 6: 419-25.

Gupta PB., Kuperwasser C., Brunet JP., Ramaswamy S., Kuo WL., Gray JW., Naber SP., & Weinberg RA. (2005). The melanocyte differentiation program predisposes to metastasis after neoplastic transformation. *Nat Genet* 37: 1047-54.

Halin S., Wikstrom P., Rudolfsson SH., Stattin P., Doll JA., Crawford SE., & Bergh A. (2004). Decreased pigment epithelium-derived factor is associated with metastatic phenotype in human and rat prostate tumors. *Cancer Res* 64: 5664-71.

Halin S., Rudolfsson SH., Doll JA., Crawford SE., Wikstrom P., & Bergh A. (2010). Pigment epithelium-derived factor stimulates tumor macrophage recruitment and is downregulated by the prostate tumor microenvironment. *Neoplasia* 12: 336-45.

Hanahan D., & Weinberg RA. (2000). The hallmarks of cancer. *Cell* 100: 57-70.

Hanahan D., & Weinberg RA. (2011). Hallmarks of cancer: the next generation. *Cell* 144: 646-74.

Haqq C., Nosrati M., Sudilovsky D., Crothers J., Khodabakhsh D., Pulliam BL., Federman S., Miller JR, 3rd., Allen RE., Singer MI., Leong SP., Ljung BM., Sagebiel RW., & Kashani-Sabet M. (2005). The gene expression signatures of melanoma progression. *Proc Natl Acad Sci U S A* 102: 6092-7.

Hase R., Miyamoto M., Uehara H., Kadoya M., Ebihara Y., Murakami Y., Takahashi R., Mega S., Li L., Shichinohe T., Kawarada Y., & Kondo S. (2005). Pigment epithelium-derived factor gene therapy inhibits human pancreatic cancer in mice. *Clin Cancer Res* 11: 8737-44.

Helfrich I., Scheffrahn I., Bartling S., Weis J., von Felbert V., Middleton M., Kato M., Ergun S., & Schadendorf D. (2010). Resistance to antiangiogenic therapy is directed by vascular phenotype, vessel stabilization, and maturation in malignant melanoma. *J Exp Med* 207: 491-503.

Helfrich I., & Schadendorf D. (2011). Blood vessel maturation, vascular phenotype and angiogenic potential in malignant melanoma: One step forward for overcoming anti-angiogenic drug resistance? *Mol Oncol*.

Hendrix MJ., Seftor EA., Hess AR., & Seftor RE. (2003). Vasculogenic mimicry and tumour-cell plasticity: lessons from melanoma. *Nat Rev Cancer* 3: 411-21.

Hendrix MJ., Seftor EA., Seftor RE., Kasemeier-Kulesa J., Kulesa PM., & Postovit LM. (2007). Reprogramming metastatic tumour cells with embryonic microenvironments. *Nat Rev Cancer* 7: 246-55.

Hess AR., Margaryan NV., Seftor EA., & Hendrix MJ. (2007). Deciphering the signaling events that promote melanoma tumor cell vasculogenic mimicry and their link to embryonic vasculogenesis: role of the Eph receptors. *Dev Dyn* 236: 3283-96.

Hoek K., Rimm DL., Williams KR., Zhao H., Ariyan S., Lin A., Kluger HM., Berger AJ., Cheng E., Trombetta ES., Wu T., Niinobe M., Yoshikawa K., Hannigan GE., & Halaban R. (2004). Expression profiling reveals novel pathways in the transformation of melanocytes to melanomas. *Cancer Res* 64: 5270-82.

Hoek KS., Schlegel NC., Brafford P., Sucker A., Ugurel S., Kumar R., Weber BL., Nathanson KL., Phillips DJ., Herlyn M., Schadendorf D., & Dummer R. (2006). Metastatic potential of melanomas defined by specific gene expression profiles with no BRAF signature. *Pigment Cell Res* 19: 290-302.

Hoek KS. (2007). DNA microarray analyses of melanoma gene expression: a decade in the mines. *Pigment Cell Res* 20: 466-84.

Hoek KS., Eichhoff OM., Schlegel NC., Dobbeling U., Kobert N., Schaerer L., Hemmi S., & Dummer R. (2008). In vivo switching of human melanoma cells between proliferative and invasive states. *Cancer Res* 68: 650-6.

Hoek KS. (2009). Melanoma progression, gene expression and DNA microarrays. *G Ital Dermatol Venereol* 144: 39-49.

Hoshina D., Abe R., Yamagishi SI., & Shimizu H. (2010). The role of PEDF in tumor growth and metastasis. *Curr Mol Med* 10: 292-5.

Hsu MY., Meier F., & Herlyn M. (2002). Melanoma development and progression: a conspiracy between tumor and host. *Differentiation* 70: 522-36.

Hutchings H., Maitre-Boube M., Tombran-Tink J., & Plouet J. (2002). Pigment epithelium-derived factor exerts opposite effects on endothelial cells of different phenotypes. *Biochem Biophys Res Commun* 294: 764-9.

Jain RK. (2003). Molecular regulation of vessel maturation. *Nat Med* 9: 685-93.

Jain RK. (2008). Lessons from multidisciplinary translational trials on anti-angiogenic therapy of cancer. *Nat Rev Cancer* 8: 309-16.

Jimenez B., & Volpert OV. (2001). Mechanistic insights on the inhibition of tumor angiogenesis. *J Mol Med* 78: 663-72.

Jubb AM., Oates AJ., Holden S., & Koeppen H. (2006). Predicting benefit from anti-angiogenic agents in malignancy. *Nat Rev Cancer* 6: 626-35.

Kajita M., McClinic KN., & Wade PA. (2004). Aberrant expression of the transcription factors snail and slug alters the response to genotoxic stress. *Mol Cell Biol* 24: 7559-66.

Kerbel RS. (2008). Tumor angiogenesis. *N Engl J Med* 358: 2039-49.

Kopp HG., Ramos CA., & Rafii S. (2006). Contribution of endothelial progenitors and proangiogenic hematopoietic cells to vascularization of tumor and ischemic tissue. *Curr Opin Hematol* 13: 175-81.

Kuphal S., Palm HG., Poser I., & Bosserhoff AK. (2005). Snail-regulated genes in malignant melanoma. *Melanoma Res* 15: 305-13.

Laquer V., Hoang V., Nguyen A., & Kelly KM. (2009). Angiogenesis in cutaneous disease: part II. *J Am Acad Dermatol* 61: 945-58; quiz 959-60.

Lawrence DA., Strandberg L., Ericson J., & Ny T. (1990). Structure-function studies of the SERPIN plasminogen activator inhibitor type 1. Analysis of chimeric strained loop mutants. *J Biol Chem* 265: 20293-301.

Levy C., Khaled M., & Fisher DE. (2006). MITF: master regulator of melanocyte development and melanoma oncogene. *Trends Mol Med* 12: 406-14.

Lin JY., & Fisher DE. (2007). Melanocyte biology and skin pigmentation. *Nature* 445: 843-50.

Loges S., Roncal C., & Carmeliet P. (2009). Development of targeted angiogenic medicine. *J Thromb Haemost* 7: 21-33.

Lomuto M., Calabrese P., & Giuliani A. (2004). Prognostic signs in melanoma: state of the art. *J Eur Acad Dermatol Venereol* 18: 291-300.

Mahabeleshwar GH., & Byzova TV. (2007). Angiogenesis in melanoma. *Semin Oncol* 34: 555-65.

Malchiodi-Albedi F., Feher J., Caiazza S., Formisano G., Perilli R., Falchi M., Petrucci TC., Scorcia G., & Tombran-Tink J. (1998). PEDF (pigment epithelium-derived factor)

promotes increase and maturation of pigment granules in pigment epithelial cells in neonatal albino rat retinal cultures. *Int J Dev Neurosci* 16: 423-32.

Maniotis AJ., Folberg R., Hess A., Seftor EA., Gardner LM., Pe'er J., Trent JM., Meltzer PS., & Hendrix MJ. (1999). Vascular channel formation by human melanoma cells in vivo and in vitro: vasculogenic mimicry. *Am J Pathol* 155: 739-52.

Matsumoto K., Ishikawa H., Nishimura D., Hamasaki K., Nakao K., & Eguchi K. (2004). Antiangiogenic property of pigment epithelium-derived factor in hepatocellular carcinoma. *Hepatology* 40: 252-9.

McGill GG., Haq R., Nishimura EK., & Fisher DE. (2006). c-Met expression is regulated by Mitf in the melanocyte lineage. *J Biol Chem* 281: 10365-73.

Meyer C., Notari L., & Becerra SP. (2002). Mapping the type I collagen-binding site on pigment epithelium-derived factor. Implications for its antiangiogenic activity. *J Biol Chem* 277: 45400-7.

Michaloglou C., Vredeveld LC., Soengas MS., Denoyelle C., Kuilman T., van der Horst CM., Majoor DM., Shay JW., Mooi WJ., & Peeper DS. (2005). BRAFE600-associated senescence-like cell cycle arrest of human naevi. *Nature* 436: 720-4.

Miller AJ., & Mihm MC, Jr. (2006). Melanoma. *N Engl J Med* 355: 51-65.

Mirochnik Y., Aurora A., Schulze-Hoepfner FT., Deabes A., Shifrin V., Beckmann R., Polsky C., & Volpert OV. (2009). Short pigment epithelial-derived factor-derived peptide inhibits angiogenesis and tumor growth. *Clin Cancer Res* 15: 1655-63.

Nguyen A., Hoang V., Laquer V., & Kelly KM. (2009a). Angiogenesis in cutaneous disease: part I. *J Am Acad Dermatol* 61: 921-42; quiz 943-4.

Nguyen DX., Bos PD., & Massague J. (2009b). Metastasis: from dissemination to organ-specific colonization. *Nat Rev Cancer* 9: 274-84.

Nico B., Benagiano V., Mangieri D., Maruotti N., Vacca A., & Ribatti D. (2008). Evaluation of microvascular density in tumors: pro and contra. *Histol Histopathol* 23: 601-7.

Notari L., Miller A., Martinez A., Amaral J., Ju M., Robinson G., Smith LE., & Becerra SP. (2005). Pigment epithelium-derived factor is a substrate for matrix metalloproteinase type 2 and type 9: implications for downregulation in hypoxia. *Invest Ophthalmol Vis Sci* 46: 2736-47.

Notari L., Baladron V., Aroca-Aguilar JD., Balko N., Heredia R., Meyer C., Notario PM., Saravanamuthu S., Nueda ML., Sanchez-Sanchez F., Escribano J., Laborda J., & Becerra SP. (2006). Identification of a lipase-linked cell membrane receptor for pigment epithelium-derived factor. *J Biol Chem* 281: 38022-37.

Nowell PC. (1976). The clonal evolution of tumor cell populations. *Science* 194: 23-8.

Ogata N., Wang L., Jo N., Tombran-Tink J., Takahashi K., Mrazek D., & Matsumura M. (2001). Pigment epithelium derived factor as a neuroprotective agent against ischemic retinal injury. *Curr Eye Res* 22: 245-52.

Orgaz JL., Martinez-Poveda B., Fernandez-Garcia NI., & Jimenez B. (2008). Following up tumour angiogenesis: from the basic laboratory to the clinic. *Clin Transl Oncol* 10: 468-77.

Orgaz JL., Ladhani O., Hoek KS., Fernandez-Barral A., Mihic D., Aguilera O., Seftor EA., Bernad A., Rodriguez-Peralto JL., Hendrix MJ., Volpert OV., & Jimenez B. (2009). 'Loss of pigment epithelium-derived factor enables migration, invasion and metastatic spread of human melanoma'. *Oncogene* 28: 4147-61.

Orgaz JL., Benguria A., Sanchez-Martinez C., Ladhani O., Volpert O., & Jimenez B. (2011). Changes in the gene expression profile of A375 human melanoma cells induced by

over-expression of multifunctional pigment epithelium-derived factor. *Melanoma Res* In Press.

Overall CM., & Lopez-Otin C. (2002). Strategies for MMP inhibition in cancer: innovations for the post-trial era. *Nat Rev Cancer* 2: 657-72.

Pavey S., Johansson P., Packer L., Taylor J., Stark M., Pollock PM., Walker GJ., Boyle GM., Harper U., Cozzi SJ., Hansen K., Yudt L., Schmidt C., Hersey P., Ellem KA., O'Rourke MG., Parsons PG., Meltzer P., Ringner M., & Hayward NK. (2004). Microarray expression profiling in melanoma reveals a BRAF mutation signature. *Oncogene* 23: 4060-7.

Pignolo RJ., Cristofalo VJ., & Rotenberg MO. (1993). Senescent WI-38 cells fail to express EPC-1, a gene induced in young cells upon entry into the G0 state. *J Biol Chem* 268: 8949-57.

Pinnix CC., & Herlyn M. (2007). The many faces of Notch signaling in skin-derived cells. *Pigment Cell Res* 20: 458-65.

Postovit LM., Seftor EA., Seftor RE., & Hendrix MJ. (2006). Influence of the microenvironment on melanoma cell fate determination and phenotype. *Cancer Res* 66: 7833-6.

Qingyi Z., Lin Y., Junhong W., Jian S., Weizhou H., Long M., Zeyu S., & Xiaojian G. (2009). Unfavorable prognostic value of human PEDF decreased in high-grade prostatic intraepithelial neoplasia: a differential proteomics approach. *Cancer Invest* 27: 794-801.

Ramirez-Castillejo C., Sanchez-Sanchez F., Andreu-Agullo C., Ferron SR., Aroca-Aguilar JD., Sanchez P., Mira H., Escribano J., & Farinas I. (2006). Pigment epithelium-derived factor is a niche signal for neural stem cell renewal. *Nat Neurosci* 9: 331-9.

Rey S., & Semenza GL. (2010). Hypoxia-inducible factor-1-dependent mechanisms of vascularization and vascular remodelling. *Cardiovasc Res* 86: 236-42.

Ria R., Reale A., Castrovilli A., Mangialardi G., Dammacco F., Ribatti D., & Vacca A. (2010). Angiogenesis and progression in human melanoma. *Dermatol Res Pract* 2010: 185687.

Rofstad EK., Henriksen K., Galappathi K., & Mathiesen B. (2003). Antiangiogenic treatment with thrombospondin-1 enhances primary tumor radiation response and prevents growth of dormant pulmonary micrometastases after curative radiation therapy in human melanoma xenografts. *Cancer Res* 63: 4055-61.

Rofstad EK., Galappathi K., & Mathiesen B. (2004). Thrombospondin-1 treatment prevents growth of dormant lung micrometastases after surgical resection and curative radiation therapy of the primary tumor in human melanoma xenografts. *Int J Radiat Oncol Biol Phys* 58: 493-9.

Sahai E., & Marshall CJ. (2003). Differing modes of tumour cell invasion have distinct requirements for Rho/ROCK signalling and extracellular proteolysis. *Nat Cell Biol* 5: 711-9.

Sahai E. (2005). Mechanisms of cancer cell invasion. *Curr Opin Genet Dev* 15: 87-96.

Sanz-Moreno V., Gadea G., Ahn J., Paterson H., Marra P., Pinner S., Sahai E., & Marshall CJ. (2008). Rac activation and inactivation control plasticity of tumor cell movement. *Cell* 135: 510-23.

Sasaki Y., Naishiro Y., Oshima Y., Imai K., Nakamura Y., & Tokino T. (2005). Identification of pigment epithelium-derived factor as a direct target of the p53 family member genes. *Oncogene* 24: 5131-6.

Seftor EA., Meltzer PS., Schatteman GC., Gruman LM., Hess AR., Kirschmann DA., Seftor RE., & Hendrix MJ. (2002). Expression of multiple molecular phenotypes by aggressive melanoma tumor cells: role in vasculogenic mimicry. *Crit Rev Oncol Hematol* 44: 17-27.

Seftor EA., Brown KM., Chin L., Kirschmann DA., Wheaton WW., Protopopov A., Feng B., Balagurunathan Y., Trent JM., Nickoloff BJ., Seftor RE., & Hendrix MJ. (2005). Epigenetic transdifferentiation of normal melanocytes by a metastatic melanoma microenvironment. *Cancer Res* 65: 10164-9.

Seftor EA., Meltzer PS., Kirschmann DA., Margaryan NV., Seftor RE., & Hendrix MJ. (2006). The epigenetic reprogramming of poorly aggressive melanoma cells by a metastatic microenvironment. *J Cell Mol Med* 10: 174-96.

Shackleton M., & Quintana E. (2010). Progress in understanding melanoma propagation. *Mol Oncol* 4: 451-7.

Simonovic M., Gettins PG., & Volz K. (2001). Crystal structure of human PEDF, a potent anti-angiogenic and neurite growth-promoting factor. *Proc Natl Acad Sci U S A* 98: 11131-5.

Slingluff CL, Jr.., Vollmer RT., Reintgen DS., & Seigler HF. (1988). Lethal "thin" malignant melanoma. Identifying patients at risk. *Ann Surg* 208: 150-61.

Steele FR., Chader GJ., Johnson LV., & Tombran-Tink J. (1993). Pigment epithelium-derived factor: neurotrophic activity and identification as a member of the serine protease inhibitor gene family. *Proc Natl Acad Sci U S A* 90: 1526-30.

Streck CJ., Zhang Y., Zhou J., Ng C., Nathwani AC., & Davidoff AM. (2005). Adeno-associated virus vector-mediated delivery of pigment epithelium-derived factor restricts neuroblastoma angiogenesis and growth. *J Pediatr Surg* 40: 236-43.

Takenaka K., Yamagishi S., Jinnouchi Y., Nakamura K., Matsui T., & Imaizumi T. (2005). Pigment epithelium-derived factor (PEDF)-induced apoptosis and inhibition of vascular endothelial growth factor (VEGF) expression in MG63 human osteosarcoma cells. *Life Sci* 77: 3231-41.

Tammela T., & Alitalo K. (2010). Lymphangiogenesis: Molecular mechanisms and future promise. *Cell* 140: 460-76.

Taniwaki T., Becerra SP., Chader GJ., & Schwartz JP. (1995). Pigment epithelium-derived factor is a survival factor for cerebellar granule cells in culture. *J Neurochem* 64: 2509-17.

Tatzel J., Poser I., Schroeder J., & Bosserhoff AK. (2005). Inhibition of melanoma inhibitory activity (MIA) expression in melanoma cells leads to molecular and phenotypic changes. *Pigment Cell Res* 18: 92-101.

Tombran-Tink J., & Johnson LV. (1989). Neuronal differentiation of retinoblastoma cells induced by medium conditioned by human RPE cells. *Invest Ophthalmol Vis Sci* 30: 1700-7.

Tombran-Tink J., Shivaram SM., Chader GJ., Johnson LV., & Bok D. (1995). Expression, secretion, and age-related downregulation of pigment epithelium-derived factor, a serpin with neurotrophic activity. *J Neurosci* 15: 4992-5003.

Tombran-Tink J., Aparicio S., Xu X., Tink AR., Lara N., Sawant S., Barnstable CJ., & Zhang SS. (2005). PEDF and the serpins: phylogeny, sequence conservation, and functional domains. *J Struct Biol* 151: 130-50.

Tombran-Tink J. (2010). PEDF in angiogenic eye diseases. *Curr Mol Med* 10: 267-78.

Topczewska JM., Postovit LM., Margaryan NV., Sam A., Hess AR., Wheaton WW., Nickoloff BJ., Topczewski J., & Hendrix MJ. (2006). Embryonic and tumorigenic pathways converge via Nodal signaling: role in melanoma aggressiveness. *Nat Med* 12: 925-32.

Tresini M., Pignolo RJ., Allen RG., & Cristofalo VJ. (1999). Effects of donor age on the expression of a marker of replicative senescence (EPC-1) in human dermal fibroblasts. *J Cell Physiol* 179: 11-7.

Uong A., & Zon LI. (2010). Melanocytes in development and cancer. *J Cell Physiol* 222: 38-41.

Utikal J., Schadendorf D., & Ugurel S. (2007). Serologic and immunohistochemical prognostic biomarkers of cutaneous malignancies. *Arch Dermatol Res* 298: 469-77.

Villanueva J., & Herlyn M. (2008). Melanoma and the tumor microenvironment. *Curr Oncol Rep* 10: 439-46.

Volpert OV. (2000). Modulation of endothelial cell survival by an inhibitor of angiogenesis thrombospondin-1: a dynamic balance. *Cancer Metastasis Rev* 19: 87-92.

Volpert OV., Zaichuk T., Zhou W., Reiher F., Ferguson TA., Stuart PM., Amin M., & Bouck NP. (2002). Inducer-stimulated Fas targets activated endothelium for destruction by anti-angiogenic thrombospondin-1 and pigment epithelium-derived factor. *Nat Med* 8: 349-57.

Weeraratna AT. (2005). A Wnt-er wonderland--the complexity of Wnt signaling in melanoma. *Cancer Metastasis Rev* 24: 237-50.

Xi Y., Nakajima G., Hamil T., Fodstad O., Riker A., & Ju J. (2006). Association of insulin-like growth factor binding protein-3 expression with melanoma progression. *Mol Cancer Ther* 5: 3078-84.

Xu X., Zhang SS., Barnstable CJ., & Tombran-Tink J. (2006). Molecular phylogeny of the antiangiogenic and neurotrophic serpin, pigment epithelium derived factor in vertebrates. *BMC Genomics* 7: 248.

Yabe T., Wilson D., & Schwartz JP. (2001). NFkappaB activation is required for the neuroprotective effects of pigment epithelium-derived factor (PEDF) on cerebellar granule neurons. *J Biol Chem* 276: 43313-9.

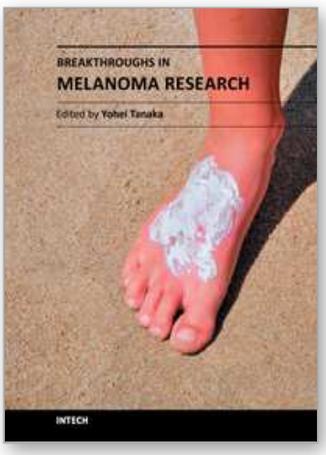
Yamagishi S., Inagaki Y., Nakamura K., Abe R., Shimizu T., Yoshimura A., & Imaizumi T. (2004). Pigment epithelium-derived factor inhibits TNF-alpha-induced interleukin-6 expression in endothelial cells by suppressing NADPH oxidase-mediated reactive oxygen species generation. *J Mol Cell Cardiol* 37: 497-506.

Yang H., & Grossniklaus HE. (2010). Constitutive overexpression of pigment epithelium-derived factor inhibition of ocular melanoma growth and metastasis. *Invest Ophthalmol Vis Sci* 51: 28-34.

Yasui N., Mori T., Morito D., Matsushita O., Kourai H., Nagata K., & Koide T. (2003). Dual-site recognition of different extracellular matrix components by anti-angiogenic/neurotrophic serpin, PEDF. *Biochemistry* 42: 3160-7.

Zaichuk TA., Shroff EH., Emmanuel R., Filleur S., Nelius T., & Volpert OV. (2004). Nuclear factor of activated T cells balances angiogenesis activation and inhibition. *J Exp Med* 199: 1513-22.

Zbytek B., Carlson JA., Granese J., Ross J., Mihm MC., & Slominski A. (2008). Current concepts of metastasis in melanoma. *Expert Rev Dermatol* 3: 569-585.



Breakthroughs in Melanoma Research

Edited by Dr Yohei Tanaka

ISBN 978-953-307-291-3
Hard cover, 628 pages
Publisher InTech
Published online 30, June, 2011
Published in print edition June, 2011

Melanoma is considered to be one of the most aggressive forms of skin neoplasms. Despite aggressive researches towards finding treatments, no effective therapy exists to inhibit the metastatic spread of malignant melanoma. The 5-year survival rate of metastatic melanoma is still significantly low, and there has been an earnest need to develop more effective therapies with greater anti-melanoma activity. Through the accomplishment of over 100 distinguished and respected researchers from 19 different countries, this book covers a wide range of aspects from various standpoints and issues related to melanoma. These include the biology of melanoma, pigmentations, pathways, receptors and diagnosis, and the latest treatments and therapies to make potential new therapies. Not only will this be beneficial for readers, but it will also contribute to scientists making further breakthroughs in melanoma research.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen