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The effects of PEDF on cancer biology: mechanisms of action and therapeutic potential

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Abstract

The potent actions of pigment epithelium-derived factor (PEDF) on tumour-associated cells, and its extracellular localization and secretion, stimulated research on this multifunctional serpin. Such studies have identified several PEDF receptors and downstream signalling pathways. Known cellular PEDF responses have expanded from the initial discovery that PEDF induces retinoblastoma cell differentiation to its anti-angiogenic, antitumorigenic and antimetastatic properties. Although the diversity of PEDF activities seems to be complex, they are consistent with the varied mechanisms that regulate this multimodal factor. If PEDF is to be used for cancer management, a deeper appreciation of its many functions and mechanisms of action is needed.

Pigment epithelium-derived factor (PEDF; encoded by *SERPINF1* and also known as EPC1 and caspin), is a serpin that has multiple biological actions. The era of PEDF research began around 1990 with the discovery that PEDF is a differentiation factor for retinoblastoma cells[1,2]. The PEDF protein was isolated from media that was conditioned by cultured retinal pigment epithelial cells, hence its name. Soon after, it was reported that expression of *SERPINF1* is increased in quiescent young fibroblasts and is specifically associated with G_0 growth arrest: PEDF expression levels are negligible in senescent fibroblasts[3,4]. Moreover, PEDF levels decline during ageing, and its expression is used as a marker for young cells[5–7]. About a decade after its discovery, PEDF was found to be a potent inhibitor of angiogenesis[8]. This finding, along with the fact that ageing is the major risk factor for the development of several different types of cancer — age-related changes in the tissue microenvironment facilitate tumour growth[9,10] — provided the impetus to study the mechanisms of action and regulation of PEDF, and its possible applications to cancer therapy. This has led to the identification of PEDF as a major antagonist to angiogenic factors (such as vascular endothelial growth factor (VEGF)), evidence for its antitumorigenic and antimetastatic activities, and its potential use as a diagnostic and prognostic marker for cancer management.

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Competing interests statement

The authors declare competing financial interests. See Web version for details.

DATABASES

National Cancer Institute Drug Dictionary: http://www.cancer.gov/drugdictionary bevacizumab | dexamethasone | tamoxifen

FURTHER INFORMATION

S. Patricia Becerra's homepages: http://irp.nih.gov/pi/s-patricia-becerra; http://www.nei.nih.gov/intramural/protein_struct func.asp

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In this Review, we discuss some of the new insights into the action of PEDF on tumours, and we focus on emerging concepts and the mechanisms of action and regulation of PEDF. We summarize the evidence that the diminishing levels of PEDF observed in various tumour types may account, at least in part, for increased malignant characteristics during tumour progression. We briefly discuss recent reports on PEDF involvement in lipid metabolism and its relevance to cancer. Finally, given that the potential use of PEDF in cancer therapeutics has generated much expectation, we also examine reports on the development of promising PEDF delivery systems.

PEDF biochemistry

As a member of the serine protease inhibitor (serpin) superfamily, PEDF belongs to a group of proteins that have a common three-dimensional structure[1,11]. The three-dimensional structure of human PEDF has been determined by X-ray crystallography[12] (Protein Data Bank (PDB) identifier 1IMV) and shows that the protein folds like a serpin. Most serpins, such as antitrypsin, antichymotrypsin and antithrombin, are serine protease inhibitors whereas others, such as ovalbumin, angiotensinogen and maspin, do not have demonstrable protease inhibitory properties[13–15]. PEDF does not undergo the stressed to relaxed conformational transition that is characteristic of active serpins, and does not have demonstrable serine protease inhibitory activity[16]. Thus, it is a member of the subgroup of non-inhibitory serpins that are thought to have lost their protease inhibitory activity but have gained additional properties during evolution. Interestingly, like PEDF, antithrombin, angiotensinogen and maspin exhibit anti-angiogenic and antitumorigenic activities, thus suggesting that a common structural determinant among them might be crucial for their function.

SERPINF1 is localized on human chromosome 17p13.1, and it encodes a polypeptide of 418 amino acids that includes an amino-terminal secretion signal peptide, one *N*-glycosylation site at Asn285 (in the sequence NLT) and a serpin signature sequence YHLNQPFIFVL that ends at amino acid 398[1,11,17]. Most cells express PEDF transcripts, and the mature gene product is mainly secreted as a soluble monomeric glycoprotein that has an apparent molecular weight of ~50,000 daltons, a molecular radius (Stokes radius) <3.05 nm and an amino-terminal sequence starting at amino acid position 21 of the precursor polypeptide. PEDF is biologically active at 1–100 nM, depending on the assay. It is found extracellularly in blood, the interphotoreceptor matrix (IPM), vitreous humour and aqueous humour of the eye, cerebrospinal fluid, tears and other body fluids at physiologically relevant concentrations[18–22]. Its biological activities are thought to depend on its interactions with cell-surface receptors: PEDF receptor (PEDFR; encoded by *PNPLA2* and is also known as desnutrin, ATGL and iPLA2ζ), laminin receptor, F_1 ATPase/synthase and low-density lipoprotein receptor-related protein 6 (LRP6) (FIG 1,FIG 2). It also has binding affinity for extracellular matrix (ECM) components heparin, heparan sulphate, hyaluronan and collagens[23–25]. The amino acids that are crucial for these interactions have been mapped on human PEDF; these are basic amino acids for heparin binding (Lys146, Lys147 and Arg149) and for hyaluronan binding (Lys189, Lys191, Arg194 and Lys197), and acidic amino acids for collagen binding (Asp256, Asp258 and Asp300)[24,26].

Segmentation of the PEDF polypeptide by chemical proteolysis and recombinant DNA technology provided much of the information that has been accumulated to date on the structure–function relationships of PEDF. Most proteinases cleave PEDF at its homologous serpin reactive loop, leaving a core polypeptide that retains the anti-angiogenic, differentiating and neurotrophic activities of the protein, as well as its affinity for ECM components[16,23,25]. More importantly, when PEDF is truncated from its carboxy-terminal end, such as bacterially expressed BH (Asp44–Pro418), BP (Asp44–Pro267), BX (Asp44–

Leu228) and BA (Asp44–Thr121) fragments, it retains its neuronal-differentiating and survival activities in retinoblastoma cells, cerebellar granule cells and motor neurons[16,27–31]. Synthetic peptides based on the smallest BA region — a 34 amino acid peptide (Asp44–Asn77) and a 44 amino acid peptide (Val78–Thr121) — exhibit anti-angiogenic and differentiating activities, respectively[32–35]. A shorter peptide derived from the 34 amino acid peptide designated P18 (Asn60–Asn77) is more effective in blocking angiogenesis than the parental 34-mer peptide, and P18 inhibits the growth of prostate and renal tumour xenografts[36].

PEDF and its relevance to cancer

The major biological responses to PEDF observed *in vitro* and *in vivo* are summarized in TABLE 1. These observations show that PEDF has been implicated in diverse biological processes, such as neurogenesis, neuroprotection, anti-angiogenesis, retina protection, stem cell renewal and inflammation. One prominent area of interest is the emerging anticancer role for PEDF. The strongest support for this role comes from the findings that PEDF exhibits anti-angiogenic and antimetastatic activities. Moreover, the exogenous administration of PEDF to bolster the declining intratumoural levels of PEDF during tumour progression results in the inhibition of tumour growth and prolonged survival in various animal models (TABLE 2). It is important to note that the functions and mechanisms of PEDF often act in opposition to protumorigenic processes (FIG. 3).

From neuronal differentiation to inhibiting tumour growth

The discovery of PEDF was based on the fact that it promotes the differentiation of human Y-79 and Weri retinoblastoma cells[1,37,38]. PEDF promotes neurite outgrowth from these cells with concomitant increases in the expression of neuronal markers such as neuron-specific enolase and neurofilament proteins. The ability of PEDF to promote the differentiation of retinoblastoma cells and other tumour cells of neuronal origin provided the first suggestion that PEDF could act directly on tumours and reduce their malignant phenotype. In this regard, PEDF-treated retinoblastoma cells are less tumorigenic than untreated controls, as shown by their delayed formation of tumours in rat retinas[39]. In the brain, Schwann cells that naturally secrete PEDF induce the differentiation of neuroblastoma cells to a less-malignant phenotype, and also inhibit tumour angiogenesis, thereby demonstrating the multifunctional antitumorigenic action of PEDF *in vivo*[40]. PEDF also causes neuroendocrine differentiation in prostate cancer cells, in addition to inhibiting tumour angiogenesis[35]. More recently, it was demonstrated that PEDF inhibits the number and proliferation of brain metastases of breast cancer cells and concomitantly protects neurons close to the metastases from cell death[41], thus highlighting its role as a double agent in limiting brain metastases and their local consequences. The ability of PEDF to induce, perhaps simultaneously, growth arrest, tumour cell differentiation to a less-malignant phenotype and protection of normal neuronal cells requires a complex system of regulation.

From inhibition of ocular neovascularization to tumour anti-angiogenesis

In the same year that the US National Cancer Institute designated the development of anti-angiogenic therapies for cancer a national priority, Dawson and co-workers[8] identified PEDF as a potent inhibitor of angiogenesis in the eye. They showed that under physiological conditions PEDF is responsible for the avascularity of the IPM and the vitreous and aqueous humour, which is crucial for visual function. Remarkably, the concentrations of PEDF in the eye are inversely correlated with ocular angiogenic development[42–44], and PEDF overexpression or local protein delivery prevent ocular neovascularization and delay photo-receptor and neural retinal cell death *in vivo* [33,40,45–50]. These findings clearly show that PEDF is an endo-genous inhibitor of angiogenesis and protects the eye. Together with the

detection of PEDF in several other tissues, these observations led researchers to explore the anti-angiogenic properties of PEDF on tumours.

Numerous studies in a variety of models have shown the anti-angiogenic effects that PEDF has on tumours (TABLE 2). An increasing number of studies are confirming the initial hypothesis that decreased PEDF expression is one mechanism driving tumour growth. For example, the growth of human Wilms' tumour xenografts in mice is suppressed by PEDF[51]. PEDF expression is high in normal mouse and human kidneys but is significantly decreased in Wilms' tumours. Systemic administration of PEDF suppresses tumour growth by targeting not only the tumour-associated vasculature, but also the tumour cells[52]. In another example, PEDF overexpression using lentiviral vectors blocks tumour angiogenesis in an animal model of pancreatic adenocarcinoma, leading to the proposal that PEDF gene therapy may provide a new treatment approach[53]. Similarly, augmenting intratumoural PEDF levels using adenoviral and adeno-associated viral vectors that are engineered to express PEDF inhibits tumour growth in a hepatoblastoma xenograft model by reducing angiogenesis and decreasing VEGF expression[54]. PEDF overexpression also inhibits orthotopic osteo-sarcoma growth, angiogenesis and metastasis[55]. The negative actions of PEDF on primary melanoma tumour xenografts are associated mostly with inhibition of the angiogenic response and the subsequently decreased microvessel density (MVD) in the tumours[56,57]. In this model, the inhibition of melanoma growth is through the inhibition of angiogenesis[58]. Furthermore, *in vitro*, PEDF inhibits melanoma cell growth by inducing apoptotic cell death[58]. PEDF inhibits the growth of heterotopic SO-Rb50 retinoblastoma xenografts in mice also through its anti-angiogenic activity, whereby it decreases both MVD and VEGF expression[59]. Likewise, PEDF is downregulated in human cervical carcinoma nests compared with either the normal cervical epithelium or the non-neoplastic peritumoural epithelium[60]. The intraperitoneal injection of recombinant PEDF in mice with cervical carcinoma xenografts suppresses tumour growth. This is associated with decreases in MVD and VEGF expression levels and with the inhibition of proliferation and the induction of apoptosis in endothelial cells[60]. In another report, PEDF suppressed angiogenesis and the growth of gastric carcinoma in a xenograft model by downregulating hypoxia-inducible factor 1α (HIF1α) and VEGF[61]. It has been recently demonstrated that the combination of PEDF with radiotherapy increases the antitumour efficacy of each individual agent in an animal model of nasopharyngeal carcinoma[62]. In this system, the simultaneous treatment with PEDF and radiation has an additive effect on the downregulation of VEGF expression and on angio-genesis inhibition. Furthermore, PEDF overexpression by adeno-associated virus-mediated gene transfer inhibits Lewis lung carcinoma growth and inhibits metastasis in a mouse model of colorectal peritoneal carcinomatosis by decreasing MVD and increasing tumour cell apoptosis[63,64].

The anti-angiogenic effect of PEDF is associated with disruption of the intratumoural vascular network. PEDF blocks the formation of endothelial capillary-like networks in culture and vessel sprouting from chicken aortic rings *ex vivo* [33,65]. Interestingly, PEDF selectively induces endothelial cell apoptosis in actively remodelling vessels rather than mature, existing ones[66,67]. A remarkable characteristic of PEDF is its potent anti-angiogenic activity compared with other endogenous inhibitors of neovascularization, such as thrombospondin, angiostatin or endostatin[8], which makes PEDF an excellent candidate for drug development. PEDF is thought to interact with specific receptors on the surface of endothelial cells to trigger anti-angiogenic signalling pathways that alter gene expression (FIG. 2). In summary, increasing the levels of PEDF in a number of animal models resulted in suppression of tumorigenesis, which was mediated mainly by inhibition of intratumoural neovascularization and endothelial proliferation, and by promotion of endothelial cell apoptosis and downregulation of pro-angiogenic factors.

Direct effects of PEDF on tumour cell death and proliferation

As noted in some of the studies discussed above, in addition to its differentiating and anti-angiogenic activities, PEDF exerts direct effects on tumour growth *in vivo*. In culture, PEDF can inhibit proliferation and promote apoptosis of tumour cells. As highlighted in a report by Doll and co-workers[47], exogenous recombinant PEDF induces tumour epithelial cell apoptosis *in vitro* and limits the growth of prostate tumour xenografts *in vivo* by simultaneously causing extensive necrosis and significantly reducing the proliferation of cells in the remaining viable tumour regions. Overexpression of PEDF using adenoviral vectors can also inhibit the proliferation and augment the apoptosis of PC-3 prostate carcinoma cells, in comparison with control cells[68]. Similarly, a proportion of cultured glioma cells undergo cell death after exposure to PEDF *in vitro*, in a dose-dependent manner[69]. Moreover, prevention of cell growth and induction of apoptosis by overexpressing PEDF in U251 glioma cells was accompanied by thrombospondin upregulation, VEGF and fibroblast growth factor 2 (FGF2) downregulation, and a lower production of matrix metalloproteinase 9 (MMP9) relative to control cells[70]. Consistently, PEDF-induced apoptosis is associated with increased levels of p53 and BAX, and the concomitant inhibition of BCL-2. *In vivo*, xenografts of PEDF-transfected U251 glioma cells, which also undergo apoptosis, are significantly smaller than controls that do not express PEDF. These findings, along with the observed loss of PEDF expression during glioma progression, point to the potential of PEDF as a treatment for patients with malignant gliomas. Apoptosis is also detected in other xenograft models in which PEDF is overexpressed, such as mouse colorectal peritoneal carcinomatosis and Lewis lung carcinoma[63,64,71]. Recombinant PEDF also induced apoptosis of osteosarcoma SaOS-2 cells *in vitro*, and in mice it reduced the growth of SaOS-2 tibial tumours and the number of lung macrometastases[72]. The negative effects of PEDF on tumour cell viability have also been demonstrated using breast cancer cells *in vitro* and through direct intracranial implantation to model metastasis[41]. Konson and co-workers[73] compared the rate of apoptosis in PEDF-treated MDA-MB-231, HCT116 and U87-MG tumour cells to that of PEDF-treated BAEC and HUVEC endothelial cells and observed that the apoptosis-inducing efficacy of PEDF in culture is stronger on endothelial cells relative to tumour cells.

PEDF and inhibition of tumour cell invasion and metastasis

Metastasis remains the cause of death for most cancer patients. The ability of PEDF to suppress tumour cell invasion and migration has been described *in vitro* and in several metastasis models *in vivo*. For example, it has been shown that PEDF can reduce the invasiveness of UMR 106–01 and SaOS-2 osteosarcoma cells, and can suppress the development of macroscopic pulmonary metastasis in an orthotopic human osteosarcoma model[55,74]. Like full length PEDF, human PEDF-derived peptides markedly increased osteosarcoma cell adhesion to type-1 collagen (collagen I) and significantly inhibited invasion[75]. Similarly, the addition of PEDF increased the adhesion of chondrosarcoma cells to surfaces coated with collagen I and, consistently, decreased cell invasion through collagen I gels[76]. The authors proposed that PEDF is a clinically appealing drug for the treatment of connective-tissue cancers, such as osteosarcoma and chondrosarcoma, and suggested the possibility of developing short PEDF peptide fragments as therapeutic tools.

Another example is in brain metastases of breast cancer origin, which are a substantial cause of morbidity and mortality for patients with breast cancer. Because PEDF is downregulated in these metastases compared with primary breast tumours, Fitzgerald and co-workers[41] explored the possible benefits of restoring PEDF to higher expression levels to limit the metastatic potential of breast cancer cells. They showed that PEDF overexpression decreases the metastasis of human and mouse breast cancer cells to the brain, in a rapid and angiogenesis-independent manner. The exciting observation was that PEDF also exhibits its

known survival effect on the neurons that shielded the brain from tumour-induced damage. In culture, PEDF exhibits potent antimigratory activity on human breast tumour cells[77,78] and neuroprotectant activity on neurons. These observations emphasize the dual role of PEDF as both a metastatic suppressor and a neuroprotectant in the brain.

The inverse association between PEDF expression levels and metastasis has been reported in studies of the invasiveness of glioma[70], metastasis to the liver from pancreatic ductal adenocarcinoma[79], progression towards a metastatic phenotype in prostate tumours[68], and liver and lung metastases of melanoma origin[57,80]. More importantly, PEDF emerged as an inhibitor of metastasis in these models both *in vivo* and *in vitro*. Lentivirus-mediated PEDF gene transfer not only decreased tumour size and intratumoural MVD, but also the number of hepatic micrometastases in a mouse model of ocular melanoma[81]. Consistently, silencing of PEDF expression in normal melanocytes and poorly aggressive melanoma cell lines increased their migration and invasiveness, which translated into an increased proliferative and *in vivo* metastatic potential[80]. It has been observed that metastatic melanoma cells adapt to facilitate metastasis by promoting a switch from a protease-dependent mesenchymal morphology to a protease-independent amoeboid phenotype[82]. Interestingly, Ladhani and co-workers[82] reported that PEDF suppresses the rounded morphology of melanoma cells and inhibits the surface localization of MMP14 (also known as MT1-MMP). This shows that PEDF blocks tumour extravasation by regulating cell shape and proteolysis, and thus identified a potential mechanism for its antimetastatic activity. Overall, these findings suggest that increasing the level of endogenous PEDF may provide an effective antimetastatic approach.

Recently, Grippo and co-workers[83] established a connection between tumour invasion, PEDF expression and lipid metabolism in the pancreas. PEDF levels are lowered in pancreatic cancers, and its expression is correlated with reduced hepatic metastases and an improved prognosis. These authors also reported an inverse correlation between PEDF and VEGF levels in human pancreatic cancer. Interestingly, PEDF has been implicated in regulating lipid metabolism and adipogenesis, both of which are known to influence pancreatic cancer progression[83–85]. In the elastase (EL)-*Kras*^{G12D} mouse model of non-invasive cystic papillary neoplasms, loss of *Serpinf1* results in the development of pancreatic ductal adenocarcinoma[83]. Moreover, these mice have increased pancreatic stromal adiposity, and the cells within the stroma express adipose markers. These findings suggest that PEDF is a crucial negative regulator of adiposity and tumour progression in the pancreas. The therapeutic implications of these findings have yet to be tested.

Molecular mechanisms of action

Although the diversity of PEDF activities may seem complex, they are consistent with the mechanisms regulating this multimodal factor. The molecular mechanisms by which PEDF functions to regulate tumour and endo-thelial cell behaviour have begun to be elucidated and are based mostly on the interactions of extracellular PEDF with different cell-surface proteins expressed by target tissues. The Boulton laboratory explored the effects of PEDF on γ-secretase, which can cleave the VEGF receptor (VEGFR)[86,87]. They showed that the addition of PEDF to microvascular endothelial cells significantly increases γ-secretase activity even in the absence of VEGF. This finding is associated with translocation of presenilin 1 from the perinuclear region to the cell membrane. The proposed mechanism for PEDF-mediated anti-angiogenesis involves cleavage at Val767 of the VEGFR1 transmembrane domain and the intracellular translocation of the carboxy-terminal fragment of VEGFR1 (FIG. 2). They also showed that PEDF upregulated presenilin 1, which facilitates the association between protein tyrosine phosphatases and VEGFR1 to inhibit VEGF-induced phosphorylation of VEGFR1. Konson and co-workers[77] reported that different

tumour-suppressive activities of PEDF are independently regulated by two different MAPK pathways: the JUN N-terminal kinase (JNK) pathway regulates the endothelial pro-apoptotic activity and the p38 MAPK pathway regulates antimigratory activities. The contributions of the Volpert laboratory elucidated how PEDF regulates CD95 ligand (CD95L; also known as FAS ligand) and cellular FLICE-like inhibitory protein (FLIP) to inhibit neovascularization in activated endothelial cells[66,88]. In these studies the authors showed that PEDF activates ERK5, which activates peroxisome proliferator-activated receptor-γ (PPARγ). This results in the increased expression of CD95L on endothelial cells[88] (FIG. 2). Expression of the essential partner of CD95L, CD95, is low in quiescent endothelial cells and vessels, but is increased by inducers of angiogenesis (such as VEGF and FGF2), thereby specifically sensitizing the stimulated endothelial cells to undergo apoptosis[66]. The anti-angiogenic activity of PEDF both *in vitro* and *in vivo* is dependent on this dual induction of CD95 and CD95L and the resulting apoptosis. Other studies have shown that VEGF and PEDF also have different effects on nuclear factor of activated T cells cytoplasmic 2 (NFATc2). NFATc2 binds to the *FLIP* promoter in the presence of VEGF and leads to the increased expression of FLIP and decreased caspase-8-mediated apoptosis[89]. Conversely, PEDF triggers JNK-mediated phosphorylation of NFATc2 and sequesters it in the cytoplasm, which prevents the NFATc2-mediated block of FLIP expression[89] (FIG. 2). It has been proposed that this coordination between pro-angiogenic factors and PEDF in the regulation of angiogenesis provides one explanation for the ability of PEDF to target remodelling capillaries for destruction. These findings indicate that PEDF probably binds to a receptor or receptors that mediate these signalling pathways in endothelial cells. Indeed, initial studies showed that endothelial cells, retinoblastoma and normal retinal cells, motor neurons and prostate tumour cells expressed receptors for PEDF, as well as for the 34-mer and 44-mer peptides of PEDF[32,34,35,90].

The first receptor identified for PEDF, PEDFR, was discovered in our laboratory[91]. PEDFR is a lipase-linked cell membrane protein that is activated on PEDF binding, and the phospholipase activity of PEDFR results in the release of free fatty acids and lysophosphatidic acids from the phospholipids in the plasma membrane[91]. PEDFR also has triglyceride lipase activity that is associated with lipid droplets in adipose tissues[92–94]. The lipid composition of the plasma membrane determines the substrate for the phospholipase, and the product determines the type of lipid mediator. For example, PEDFR activity can result in the generation of either the omega-3 fatty acid docosahexaenoic acid (DHA) or the omega-6 fatty acid arachidonic acid (AA)[94,95] (FIG. 1). DHA can reduce the invasive phenotype of human melanoma, breast and renal carcinoma cells *in vitro*, which implies that DHA might modify the tumour cell metastatic potential and be a lipid mediator of PEDF signalling. However, the mechanisms by which DHA can directly affect the invasive phenotype of cancer cells remain unclear. AA has promigratory and tumour growth properties[96], which can be antagonized by DHA[97,98]. It has been suggested that the relative levels of DHA and AA in the tumour environment might have a profound impact on tumour growth properties. In this manner, the composition of fatty acids in plasma membranes is likely to control the outcome of PEDF-mediated activation of PEDFR. DHA is a precursor of 10,17*S*-docosatriene (also known as neuroprotectin D1 (NPD1)). It is thought that an as yet unknown phospholipase acts on membrane phospholipids to free DHA that is then converted to 17*S*-H(p)DHA, oxidized by 15-lipoxygenase (15-LOX) to 16,17-epoxy-docosatriene, which is finally converted to NPD1 (REF. 99). NPD1 has been shown to have anti-angiogenic, neurotrophic and anti-inflammatory properties in the brain and retina[99,100]. Interestingly, a number of reports have shown that exogenous DHA induces cytotoxicity in a wide range of cancer cell types, which has led to the investigation of DHA in several clinical cancer trials[101,102]. DHA has been shown to suppress tumour invasion[96,103] and retinal angiogenesis[97,98], but whether this involves the PEDF pathway is not yet clear. One study using prostate cancer cells clearly shows that PEDFR is crucial for the antitumour actions of

PEDF[104]. PEDF binding to PEDFR upregulates PPARγ, which leads to the suppression of nuclear factor-κB (NF-κB)-mediated transcriptional activation, reduced production of interleukin 8 (IL-8) and limited proliferation of prostate cancer cells (FIG. 1). Although it is not yet established how PEDF–PEDFR-mediated PPARγ induction occurs, it has been shown that 5-LOX acting on DHA to produce 4-hydroxy-DHA — or DHA binding to cytosolic fatty-acid-binding protein 7 (FABP7), which has a higher affinity for DHA than for AA — translocates DHA to the nucleus where it binds to PPARγ, thus resulting in the inactivation of NF-κB and the downregulation of promigratory genes (such as cyclooxygenase 2 (*COX2*))[96,105] (FIG. 1).

Bernard and co-workers[106] reported a second receptor for PEDF, laminin receptor, in endothelial cells. PEDF binds laminin receptor through the Asp44–Asn77 region of PEDF (the 34-mer), and this interaction is linked to the anti-angiogenic functions of PEDF. PEDF is also a ligand of a third membrane protein, F_1 ATP synthase, which is expressed on the surface of endothelial and tumour cells[107]. The same 34 amino acid region of PEDF inhibits the formation of ATP from ADP and inorganic phosphate catalysed by the ATP synthase, and this limits angiogenesis and tumour cell viability[108] (FIG 1,FIG 2). More recently, Park and co-workers[109] reported that PEDF binds to LRP6, which is a WNT co-receptor, and blocks the signalling that is induced by WNT ligands in retinal pigment epithelial cells. Although WNT signalling has a role in tumorigenesis, the relevance of the PEDF–LRP6 interaction to cancer development and progression are yet to be revealed. Finally, two reports have described the inverse regulation of PEDF and the serine protease urokinase-type plasminogen activator (uPA) receptor (uPAR) in tumours, and that PEDF can downregulate uPAR expression in endothelial cells[72,110]. Yang and co-workers[110] demonstrated that the PEDF-mediated inhibition of the VEGF-induced increase in vascular permeability involves blockade of the p38 MAPK–glycogen synthase kinase 3 (GSK3)–β-catenin signalling pathway and uPAR expression. Whether PEDF binds uPAR is not yet known.

The interactions of PEDF with ECM components also affect PEDF activity. PEDF associates with collagen and glycosaminoglycans in the ECM, with binding affinities that are sensitive to pH changes[23–25,111]. At the molecular level, PEDF has distinct binding sites for collagen I and for glycosaminoglycans[24,26], which are separated from the homologous serpin reactive site. The ligand–receptor interactions of PEDF in retinoblastoma cells are positively modulated by glycosaminoglycans, such as heparin and heparan sulphate, but not by hyaluronan[112]. This suggests that glycosaminoglycans can act as cofactors of PEDF and/or its receptor to improve ligand–receptor interactions, which in turn would increase the anti-tumorigenic activity of PEDF. The involvement of the collagen-binding motif in the anti-angiogenic activity of PEDF was studied by Hosomichi and co-workers[113], who showed that a recombinant PEDF variant that is unable to bind collagen I does not inhibit tumour growth unlike wild-type PEDF and a PEDF variant with an altered heparin-binding site. The findings suggest that the collagen-binding site is involved in the anti-angiogenic and antitumour activities of PEDF.

Regulation of PEDF

Given the antiproliferative and pro-apoptotic functions of PEDF, it is not surprising that its expression and levels are downregulated and/or disrupted in cancer. The downregulation of PEDF during tumour progression has created an interest in defining the mechanisms that govern PEDF regulation and turnover for translational research. PEDF abundance and activities can be regulated both by extrinsic microenvironment-altering effectors such as hormones, vitamins, oxygenation or ECM composition, as well as by molecular drivers that alter its intrinsic properties (such as post-translational modification).

Transcriptional regulation

Studies of PEDF expression using both a rat castration model and comparative immunohistochemical analyses of biopsy specimens collected from patients before and after androgen-ablation therapy demonstrated that PEDF expression is increased in the prostate after androgen withdrawal[47]. The relevance of these results to prostate cancer treatment has yet to be fully investigated. Similarly, treatment with 17β-estradiol results in the reduced expression of PEDF mRNA and protein in ovarian surface epithelial (OSE) cells (which are possible precursors of ovarian cancer), while simultaneously promoting OSE cell and ovarian cancer cell proliferation[114].

Information on the regulation of *SERPINF1* expression by retinoids originally came from studies of the retina. In addition to their involvement in vision, retinoids have many important and diverse roles, such as regulation of cell proliferation and differentiation, growth of bone tissue, immune function and activation of tumour suppressor genes. They regulate gene transcription by binding to and activating two classes of nuclear transcription factors: the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs). The *SERPINF1* promoter has a functional retinoic-acid-responsive element (RARE), and retinoic acid upregulates *SERPINF1* expression in Y79 retino blastoma cells, endothelial and retinal pigment epithelial cells, and increases secreted PEDF protein levels, but has no effect on VEGF expression[115,116]. Consistently, the expression of PEDF in the retinal pigment epithelium is considerably lower in vitamin-A-deficient mice compared with normal control mice, and the VEGF levels remain the same[116]. These observations suggest that *SERPINF1* is a transcriptional target of retinoic acid. Recently, Doyon and co-workers[117] identified *SERPINF1* as a novel transcriptional target for the nuclear receptor co-repressor 1 (NCOR1). Studying the transition from proliferation to differentiation in intestinal epithelial cells, they showed that silencing of NCOR1 in proliferating crypt cells results in a rapid growth arrest. They also showed that the *SERPINF1* promoter is occupied by NCOR1 in proliferating epithelial cells, thereby repressing the transactivation of the *SERPINF1* promoter by RAR and RXR, and increasing cell proliferation. On forced expression of PEDF there was a slower rate of proliferation, thus demonstrating that NCOR1 is required to maintain the proliferation of epithelial cells in culture and that it reduces PEDF expression at the transcriptional level.

Similarly, PEDF is a transcriptional target of dexamethasone, which is a synthetic glucocorticoid used in cancer treatment that induces terminal differentiation in specific types of cancer. *In silico* searches of transcriptional promoter elements yield up to six glucocorticoid receptor binding sites in the transcriptional promoter of *SERPINF1*. It has been shown that dexamethasone induces *Serpinf1* mRNA expression and increases PEDF protein production in C6 rat glioma cells, in mouse Muller glial cells and in the trabecular meshwork from human eyes[115,118,119], implying that dexamethasone may act as a potential mediator of the actions of PEDF on cancer cells.

Although PEDF expression is not regulated by p53, it has been identified as a direct transcriptional target of the p63 and p73 members of the p53 family[120]. In fact, there is an association between the expression of the ΔEX2p73 variant isoform of p73 and the downregulation of PEDF in human colorectal tumours[121].

Hypoxia-mediated regulation

The degree of oxygenation in the tumour microenvironment participates in the regulation of tumour growth and can also regulate PEDF. Although hypoxia and hypoxia mimetics upregulate HIFα subunits, VEGF, MMPs and other pro-angiogenic factors, hypoxia is associated with reduced levels of PEDF[8,65]. These findings are important as they show that

hypoxia increases the angiogenic potential of the tumour by raising the ratio of pro-angiogenic factors to anti-angiogenic factors. MMPs act on the ECM to liberate VEGF that is bound to the matrix, thereby increasing the levels of VEGF that are accessible to target cells. Active MMP2 and MMP9 can also proteolyze PEDF, thus abolishing its neurotrophic and anti-angiogenic activities[65]. These results suggest that hypoxia might decrease PEDF protein levels by stimulating the MMP-mediated proteolysis of PEDF. Interestingly, the expression and secretion of VEGF, MMP2 and MMP9 positively correlate with the progression of neovascular diseases[122] and inversely correlate with PEDF levels. Several studies have explored the effects of MMP inhibitors that are used to block angiogenesis, as they should prevent the liberation of active VEGF from the ECM and maintain the levels of anti-angiogenic factors. Samtani and co-workers[123] showed in rats that oral administration of doxycycline, which is a broad-spectrum antibiotic and an MMP catalytic inhibitor, increased PEDF levels in serum and inhibited neovascularization in the retina. More recently, Fernandez-Barral and co-workers[124] reported that hypoxic conditions encountered during primary melanoma growth downregulate PEDF by a post-translational mechanism involving degradation by autophagy, and such a mechanism could therefore contribute to the highly metastatic characteristic of aggressive melanoma cells.

Post-translational modifications

Post-translational modifications can also regulate PEDF activities. Although PEDF glycosylation, amino-terminal modifications or unfolding of the PEDF protein are dispensable for biological activities[11], the Seger laboratory has shown that different phosphorylation sites can convert PEDF from a neurotrophic to an anti-angiogenic factor[125]. They prepared phosphomimetic PEDF variants with increased anti-angiogenic activities that are much more efficient than wild-type PEDF at inhibiting growth and angiogenesis in breast cancer, colon cancer and glioblastoma xenograft models[73]. Remarkably, the antitumour activity of the phosphomimetic variants is comparable to that of the established anti-angiogenic agent bevacizumab, but they act in a VEGF-independent manner, without affecting the levels of *VEGFA* mRNA or VEGF receptor 2 phosphorylation. PEDF and its variants act on intratumoural endothelial apoptosis, but in contrast to results from other groups, the Seger group reported that the variant forms do not affect the survival of cancer cells *in vitro*, hence they concluded that the anti-angiogenic activity of these agents is the main property of the observed antitumour effect. More recently, the same group reported the molecular mechanism by which phosphomimetic PEDF exerts more-profound effects at the cellular level by inducing JNK-dependent apoptosis and p38-mediated migration arrest[77]. More recently, Feng and co-workers[126] demonstrated that the triple phospho mimetic EEE-PEDF significantly reduced the growth and metastasis of choroidal melanoma xenografts in nude mice, and this effect was associated with inhibiting VEGF and NF-κB expression. Is has been proposed that EEE-PEDF has an increased negative charge compared with the wild-type protein. Interestingly, we have identified a natural PEDF protein variant with enhanced tumour cell antimigratory and cell-death-inducing activities that was separated from the canonical wild-type form by ion-exchange chromatography. This variant has increased negative charge compared with other forms of PEDF, but the chemistry of this molecule is unknown[78]. The PEDF forms with increased anticancer properties, which include the modified phosphorylated forms and the truncated peptide forms, should prove to be useful tools in the preparation of optimized PEDF molecules for therapeutic uses. Indeed, these findings encourage the development of second-generation PEDF molecules as specific, angiogenesis-targeting anticancer agents.

PEDF, cancer prognosis and therapeutic potential

In recent years it has become apparent that, in addition to the established antitumour activity of exogenously added PEDF, changes in the endogenous expression of PEDF are associated

with the malignant progression of diverse tumour types. Immunohistochemical analyses of PEDF expression in a variety of human tumour specimens and normal control tissues led to an overall picture showing that increased PEDF expression is associated with a more-favourable prognosis, whereas reduced levels of PEDF are indicative of a poorer prognosis. This general assessment has been confirmed for glioma[127], pancreatic adenocarcinoma[79], non-small-cell lung tumours[128,129], breast cancer [130,131], colorectal cancer (both in the tumours themselves[121] and in the plasma of cancer patients[132]), invasive melanoma[80], prostate cancer[133], ocular melanoma[81], clear-cell renal-cell carcinoma[134] and ovarian cancer[135]. The findings that most prostatic intra-epithelial neoplasia (PIN) specimens expressed moderate to low levels of PEDF[133] and that the levels in patient sera decrease from cases of benign prostatic hyperplasia (BPH) to those of increasing malignancy[136] suggest that PEDF losses represent an early event in carcinogenesis that becomes more acute during malignant progression. Tissue microarray studies of matched primary and recurrent breast tumours after tamoxifen treatment showed that patients who presented with progressive disease, on average 93 months after endocrine therapy, had significantly lower PEDF levels than those who showed a complete therapeutic response[137]. A poor therapeutic response correlated with the low PEDF expression levels in their primary carcinomas[137]. The fact that these studies were also correlated with *in vitro* experiments showing that modulating the levels of PEDF expression is sufficient to alter the sensitivity of breast cancer cells to endocrine therapy provides strong evidence supporting the prognostic value of PEDF for disease progression and patient outcome. Most recently, PEDF has also been identified as a prognostic marker for colorectal cancer[138]. Analyses of PEDF levels in serum samples from normal individuals and patients with cancer demonstrate that decreased PEDF levels significantly correlate with advanced clinical stage, lymph node and distant metastasis, and poorer overall survival. In agreement with previous reports[121,132], low serum PEDF levels correlate with downregulated PEDF expression in the tumours, which is also associated with disease-free survival. Nevertheless, whether changes in PEDF levels are involved in cancer onset or are a consequence of the malignant process remains to be elucidated. Although highly consistent, this picture is not perfect, and hepato-cellular carcinoma (HCC) is the most notable exception. Despite an earlier report showing that the serum concentration of PEDF was decreased in patients with cirrhosis and HCC relative to healthy individuals and to patients with chronic hepatitis[139], recent results showed that PEDF levels were higher in HCC than in adjacent normal tissues and also greater in serum samples from patients with HCC than normal controls. They also showed that the effective treatment of HCC caused significant reductions in serum PEDF levels[140]. Although this apparent discrepancy may be due to organ- or tissue-specific differences, the overwhelming evidence supports the notion that the lower the tumour PEDF levels, the poorer the prognosis and the worse the expected outcome. It is precisely for this reason that the potential use of PEDF in cancer therapeutics has generated great expectations. Consequently, substantial efforts are currently underway to optimize the use of exogenous PEDF for cancer treatment. These strategies are primarily focused in two areas: the development of protein or peptide therapeutics with enhanced antitumorigenic activity, such as PEDF phosphomimetics[73], PEDF variant forms[78] or discrete PEDF-derived peptides that recapitulate the anticancer activity of full-length PEDF[35,36]; and the establishment of efficient methods for PEDF administration that take advantage of delivery systems using either viral vectors directly[54,63,64,68,141–143], virally infected human mesenchymal stem cells[144–146], microparticles or nanoparticles of various compositions[71,147,148] or implanted micro-osmotic pumps[149]. Moreover, combination therapeutic protocols using PEDF in addition to differentiation-inducing agents such as IL-6 (REF. 150) or with radiotherapy[62] are also being studied.

Concluding remarks

Ongoing studies will further clarify the details of PEDF receptor signalling cascades and their biological importance. In particular, PEDF signalling studies may uncover new surface-to-nucleus signalling cascades that are triggered by each of the PEDF receptors. Identification of the antitumorigenic activities of PEDF *in vivo* has only recently begun, and considerably more work is required to understand mechanistically the role of PEDF in cancer. One priority is to delineate how PEDF triggers each activity in target cells and what domain of the PEDF polypeptide is responsible for a particular effect of the entire molecule. The discovery of receptors for PEDF is an exciting development as it not only underscores the importance of PEDF in tumour inhibition but also links previously unconnected areas of cell biology. Studies on the role of these receptors on cancer development and progression and their relative quantification in tumours versus normal tissues are of interest for potential therapeutic purposes. Outstanding questions concern the multifunctionality of PEDF and its regulation by influences from various cellular contexts. Defining precisely how PEDF acts within a given set of cellular and tumoural conditions to promote its diverse actions towards preventing cancer development is of great interest. As the antitumour, anti-angiogenic and antimetastatic activities of PEDF reveal a strong potential in applying PEDF treatment in the clinic, the development of efficient, safe and cost-effective delivery systems for the use of PEDF in cancer treatment should be a high-priority research area. Interestingly, no toxicity as a result of PEDF administration has been reported in any of the animal models tested. A number of reports have provided evidence in support of the use of PEDF as a prognostic factor in cancer management. Therefore, determining tumour or serum PEDF levels at diagnosis may provide an excellent source of information towards delineating more- or less-aggressive treatment protocols in order to improve the therapeutic outcome for patients with cancer. Thus, we think that PEDF will become a valuable tool in our fight against cancer.

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Glossary

Stokes radius	A molecular radius that consists of the radius of a hard sphere that diffuses at the same rate as a given molecule. A bigger molecule will have a larger Stokes radius compared with a more-compact molecule of the same molecular weight.
Interphotoreceptor matrix	(IPM). This fills the part of the eye referred to by ophthalmologists as the subretinal space. It is located between the outer limiting membrane of the retina and the apical border of the retinal pigment epithelium, where it surrounds the photoreceptor inner and outer segments that project from the outer retinal surface.
Serpin reactive loop	An exposed region in the folded protein structure of serpins that is located towards the carboxyl-terminus. It is also known as the reactive centre loop that, in inhibitory molecules, determines specificity and is involved in recognizing target proteinases.
Neurotrophic	This term is used to describe factors that promote the initial growth and development of neurons in the central nervous

	system and peripheral nervous system. They can induce the regrowth of damaged neurons.
Neovascularization	In ophthalmology, choroidal, retinal or corneal neovascularization refers to the proliferation of blood vessels and the formation of a microvasculature within the innermost layer of the choroid of the eye, inner retina or cornea, respectively.
Carcinoma nests	Cohesive aggregates of cancer cells within predominantly normal tissues.
Adiposity	Refers to the fat content of a given tissue
Trabecular meshwork	An area of tissue in the eye that is located around the base of the cornea, near the ciliary body, and is responsible for draining the aqueous humour from the eye through the chamber at the front of the eye covered by the cornea.

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At a glance

- PEDF is a member of the serpin superfamily that has many functions that often act in opposition to mechanisms that drive cancer progression.
- Tumour progression is associated with reduced levels of PEDF in tumours. Exogenous administration of PEDF to bolster the declining intratumoural levels of PEDF during tumour progression results in the inhibition of tumour growth and prolonged organismal survival in various animl models.
- PEDF can act directly on tumours to induce differentiation to a less-malignant phenotype, promote apoptotic tumour cell death and inhibit the proliferation of tumour cells.
- Numerous studies in various models have shown the anti-angiogenic effects that PEDF has on tumours. PEDF is a potent inhibitor of angiogenesis through pro-apoptotic effects on endothelial cells. It can also inhibit endothelial cell migration, endothelial tube formation, vessel sprouting and intratumoural neovascularization, and can decrease the levels of pro-angiogenic factors.
- Support for its anticancer role also comes from the findings that PEDF exhibits strong antimetastatic activity by suppressing tumour cell invasion and migration; these effects have been described *in vitro* and in several metastasis models *in vivo*.
- The molecular mechanisms by which PEDF functions to regulate tumour and endothelial cell behaviour are based mostly on its interactions with different cell-surface receptors and their downstream signalling pathways.
- PEDF abundance and activities are regulated both by extrinsic microenvironment-altering effectors (such as hormones, vitamins, oxygenation or extracellular matrix (ECM) composition), as well as by molecular drivers that alter its intrinsic properties (such as post-translational modifications).
- Numerous reports have provided evidence in support of the use of PEDF as a prognostic factor in cancer management. PEDF-positive expression is described as an independent favourable prognostic factor for cancer.

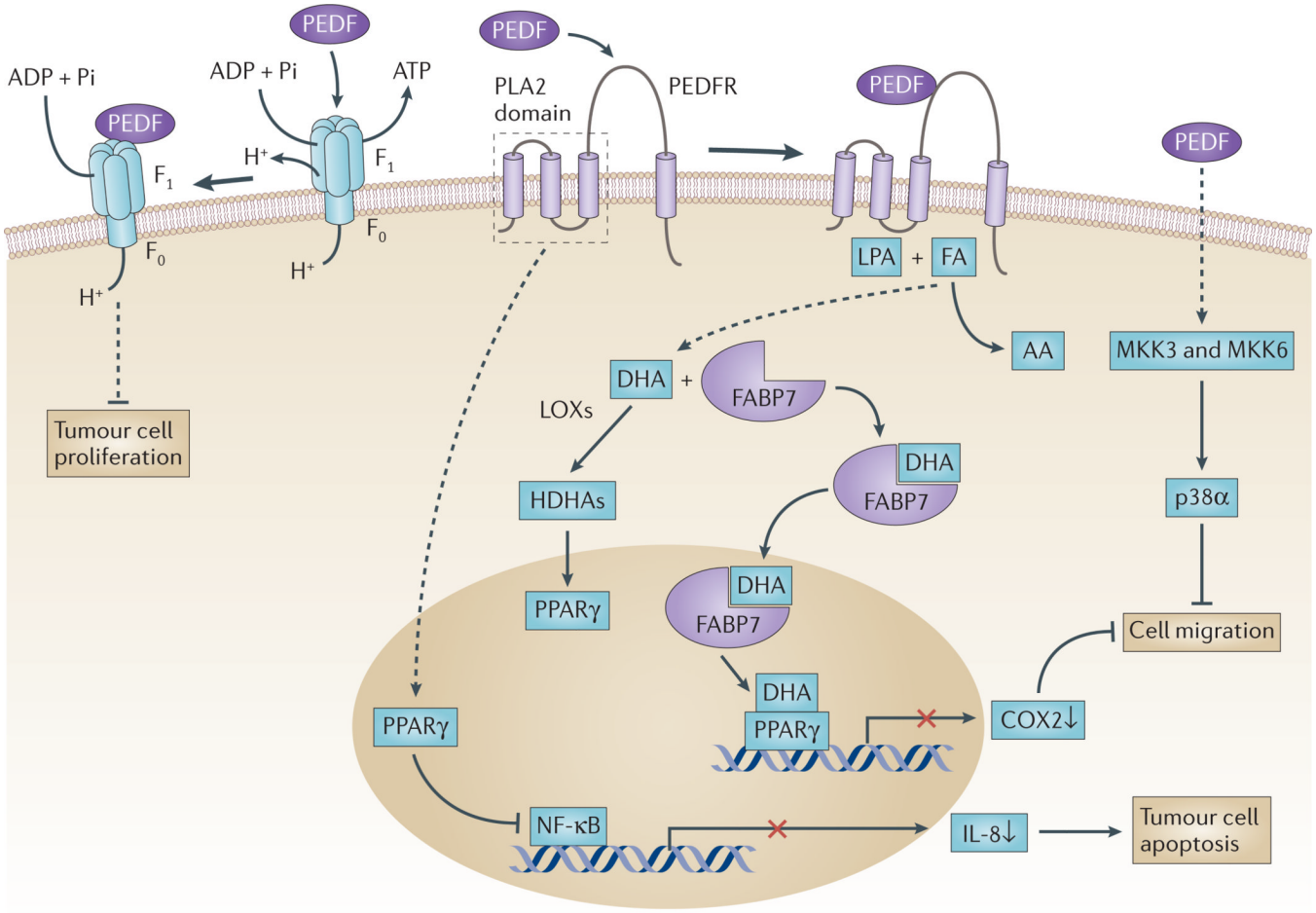

Figure 1. PEDF in tumour cells

Pigment epithelium-derived factor (PEDF) is a ligand for several receptors, and its interaction with these receptors is thought to trigger the signalling pathways illustrated here. PEDF binds to PED F receptor (PEDFR) and stimulates its phospholipase activity[91,151]. When PEDFR is at the membrane, its phospholipase A2 (PLA2) active site is located close to the phospholipid bilayer where it can use phospholipids as substrates. Depending on the relative abundance of the fatty acids omega-3 docosahexaenoic acid (DHA) and omega-6 arachidonic acid (AA) in phospholipid membranes, free DHA or AA can be liberated by PEDFR. DHA is a precursor of the anti-angiogenic and neuroprotector neuroprotectin D1 (NPD1)[152]. Other DHA metabolites, such as hydroxy-DHAs (HDHAs), which are produced by lipoxygenases (LOXs), can act on peroxisome proliferator-activated receptor-γ (PPARγ)[102,105,153]. Upregulation of PPARγ leads subsequently to the suppression of nuclear factor-κB (NF-κB)-mediated transcriptional activation, reduced production of interleukin 8 (IL-8) and limited proliferation of prostate cancer cells[104]. Cytosolic fatty-acid-binding protein 7 (FABP7) can bind DHA with higher affinity than AA, translocate DHA to the nucleus and transfer it to PPARγ, thus resulting in the downregulation of promigratory genes, such as cyclooxygenase 2 (*COX2*)[96]. PEDF is a ligand of cell-surface F ATP synthase, and its 34-mer peptide region inhibits ATP production and reduces endothelial and tumour cell viability and angiogenesis[107,108]. PEDF, through interaction with a yet unknown receptor, can sequentially activate MKK3, MKK6 and p38α MAPK to inhibit cell migration[77]. FA, fatty acid; LPA, lysophosphatidic acid.

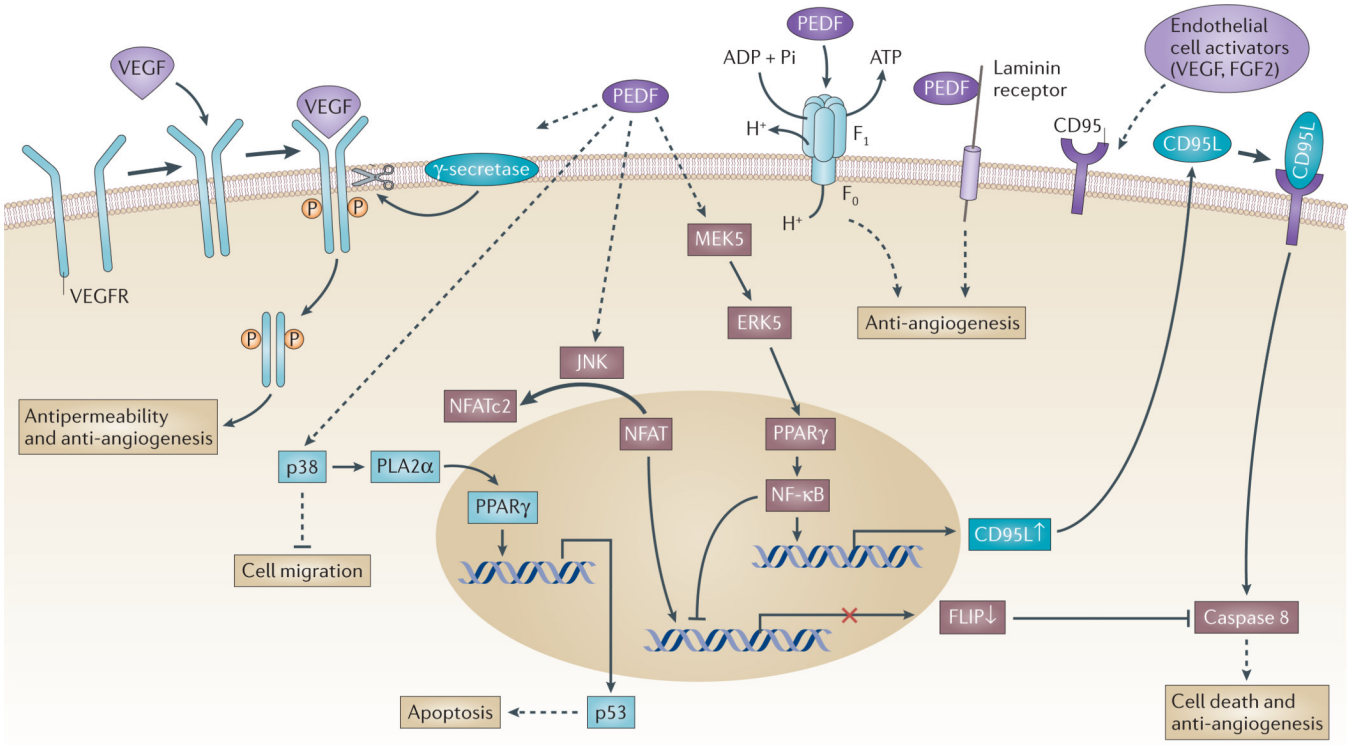

Figure 2. Signalling events of PEDF in endothelial cells

Vascular endothelial growth factor (VEGF) binds to a homodimerized VEGF receptor (VEGFR), which becomes phosphorylated and activated. Pigment epithelium-derived factor (PEDF) can increase γ-secretase-mediated cleavage of VEGFR1 and VEGFR2 at the transmembrane region to generate an intracellular domain fragment[87,154]. At the same time, PEDF can inhibit VEGF-induced phosphorylation and activation of VEGFR1 (REF. 86). PEDF inhibits VEGF-driven angiogenesis and permeability through the regulated intracellular proteolysis of VEGFR. PEDF can activate the p38 MAPK pathway to inhibit endothelial cell migration[77]. It can also activate peroxisome proliferator-activated receptor-γ (PPARγ) through cytosolic phospholipase A2α (PLA2α) to induce the expression of *TP53*, which encodes the pro-apoptotic protein p53 (REF. 155). At the same time, endothelial activators such as VEGF and fibroblast growth factor 2 (FGF2) stimulate and expose CD95 (also known as FAS) on the endothelial plasma membrane. PEDF can sequentially activate MEK5 (which is a MAPK kinase), ERK5, PPARγ and nuclear factor-κB (NF-κB), which induces the expression of the pro-apoptotic gene CD95 ligand (*CD95L*), the protein product of which translocates to the plasma membrane. The resulting CD95L-CD95 complex induces the binding and activation of caspase 8 that under certain conditions triggers the cell death cascade[66,88]. At the same time, NF-κB activation has a negative impact on cellular FLICE-like inhibitory protein (FLIP) expression, which decreases the capacity of FLIP to inhibit caspase 8. Conversely, PEDF triggers JUN N-terminal kinase (JNK)-mediated phosphorylation of nuclear factor of activated T-cells, cytoplasmic 2 (NFATc2) and sequesters it in the cytoplasm, thus blocking FLIP expression. In this manner, PEDF leads to apoptosis in activated endothelial cells. PEDF is a ligand of two known proteins on endothelial cells that result in anti-angiogenic responses. PEDF binds to laminin receptor[106] and cell-surface F1 ATP synthase to inhibit ATP production and inhibit angiogenesis[104,107,108].

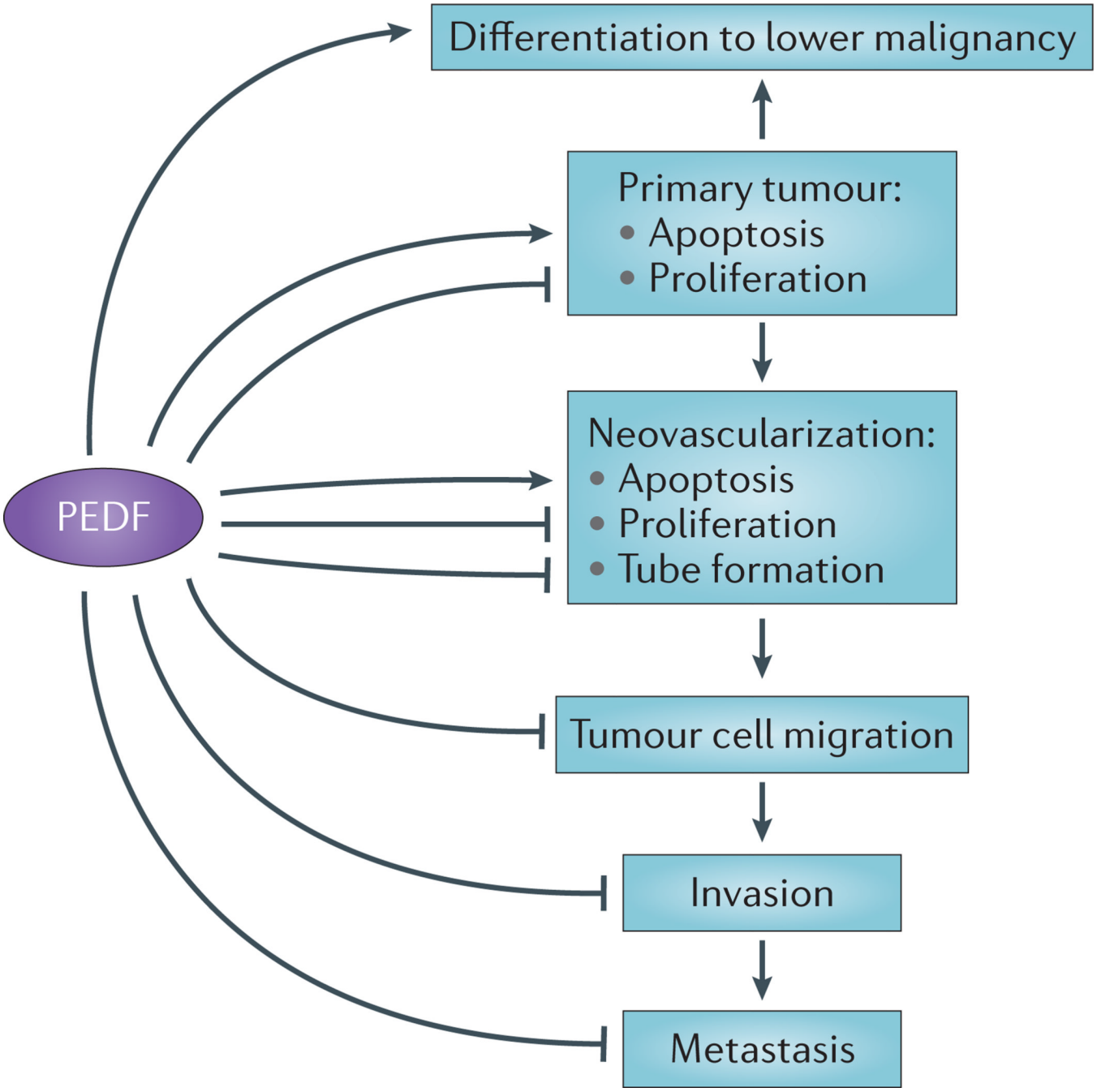

Figure 3. The effects of PEDF on tumour progression

A linear representation of different pigment epithelium-derived factor (PEDF) targets that form a concerted set of activities to inhibit cancer progression. PEDF can differentiate tumours to a less-malignant phenotype. Tumour cells secrete angiogenic factors to activate endothelial cells. PEDF can block angiogenesis-mediated activities and neovascularization. PEDF can also block tumour migration, invasion and metastasis.

Table 1

Major biological responses to PEDF

PEDF action	Target	Refs
Differentiating activity	Retinoblastoma cells, macrophages, microglia, neuroblastomas and prostate tumours	2,35,40,150,156,157
Morphogenetic	Photoreceptor cells and Muller cells	158,159
Neurite outgrowth	Retinal neurons and spinal cord motor neurons	29,160
Survival activity, anti-apoptotic	Cerebellar granule cells, motor neurons, retinal ganglion cells, photoreceptors and retinal progenitor cells	46,50,161–166
Self-renewal factor	Neural stem cells, retinal stem cells and human embryonic stem cells	167–169
Antipermeability	Retinal pigment epithelial cells and endothelial cells	110,154,170
Anti-inflammatory	Retina	171
Pro-inflammatory	Adipose tissue and macrophages	172,173
Anti-angiogenic	Ocular endothelial and tumour endothelial cells	8,33,35,36,44,54,59,60,64,71, 73,77,88,106,125,126,141,142
Induces cell death	Endothelial, tumour and glial cells	35,36,44,59,60,71,73,77,88, 106,126,142

PEDF, pigment epithelium-derived factor.

Table 2

Cancers that respond to PEDF

Tumour type	PEDF action	Refs
Retinoblastoma	• Neuronal differentiating • Anti-angiogenic • Decreased MVD • Decreased VEGF expression • Delayed tumour growth	1,2,39,59
Neuroblastoma	• Differentiation to a less-malignant phenotype • Anti-angiogenic	40,72,174
Prostate	• Anti-angiogenic • Neurocrine differentiation • Inhibition of tumour cell proliferation • Tumour epithelial cell apoptosis • Antimetastatic • Increased macrophage density	35,47,68,104,157,175
Melanoma	• Anti-angiogenic • Decreased tumour MVD • Antimetastatic	56,57,80,82,176
Wilms' tumour	• Anti-angiogenic • Tumour cell apoptosis	51,52
Pancreatic adenocarcinoma	• Anti-angiogenic • Tumour epithelial cell apoptosis • Antimetastatic (for liver metastases) • Association with prolonged patient survival • Lower adiposity	47,53,79,83
Hepatoblastoma	• Anti-angiogenic • Decreased VEGF expression	54
Osteosarcoma	• Anti-angiogenic • Antimetastatic • Inhibition of tumour cell invasiveness	55,74,75,149,177,178
Chondrosarcoma	• Inhibition of tumour cell invasiveness • Tumour cell apoptosis	76
Human cervical carcinoma	• Decreased tumour MVD • Decreased VEGF expression • Inhibition of endothelial cell proliferation	60

Tumour type	PEDF action	Refs
	• Induction of endothelial cell apoptosis	
Gastric carcinoma	• Anti-angiogenic • Downregulation of HIF1αand VEGF	61
Nasopharyngeal carcinoma	• Anti-angiogenic • Decreased VEGF expression	62
Lewis lung carcinoma	• Reduction of tumour growth • Antimetastatic • Prolonged survival of mice • Decreased tumour MVD • Tumour cell apoptosis	63,144
Colorectal peritoneal carcinoma	• Inhibition of tumour growth • Anti-angiogenic • Decreased tumour MVD • Tumour cell apoptosis • Inhibition of metastasis	64,71,73
Glioma	• Decreased tumour growth • Tumour cell death • Tumour cell apoptosis • Inhibition of tumour cell invasiveness • Anti-angiogenic	47,69,70,73,146
Uveal melanoma	• Decreased primary tumour size • Antimetastatic (for liver metastases) • Reduced proliferation, migration and invasion • Pro-apoptotic	81,126
Breast cancer xenograft	• Anti-angiogenic • Antimigratory	73,77
Brain metastasis from breast tumours	• Cell death • Antimetastatic (for brain metastases) • Neurotrophic for brain neurons in the tumour vicinity	41,78

HIF1α, hypoxia-inducible factor 1α; MVD, microvessel density; PEDF, pigment epithelium-derived factor; VEGF, vascular endothelial growth factor.