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Review

The Role of PEDF in Pancreatic Cancer

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Abstract. *Pigment epithelium-derived factor (PEDF) is an important antiangiogenic and antitumorigenic factor in a variety of cancer forms, including pancreatic cancer. PEDF is mainly secreted as a soluble monomeric glycoprotein. In human pancreatic cancer PEDF levels are decreased, both in the tissue and serum. The decrease is associated with increased tumor angiogenesis, fibrosis, inflammation, autophagy, occurrence of liver metastasis and worse prognosis. In murine models, loss of PEDF is sufficient to induce invasive carcinoma and this phenotype is associated with large lesions characterized by poor differentiation. Lentiviral gene transfer of PEDF has resulted in decreased microvessel density and has inhibited tumor growth. Herein we review the multifunctional role of PEDF in pancreatic cancer and its therapeutic potential.*

Pancreatic cancer is the 10th most common type of cancer in terms of incidence, but ranks as the third leading cause of death from cancer (1). By 2030, pancreatic cancer is projected to become the second most common cause of cancer deaths, after lung cancer (2). This trend is largely attributed to the limited progress in early detection and effective treatment, as well as to an increasing incidence, due to the ageing population (3). The 5-year survival rate for pancreatic cancer is 8% at most, representing the lowest rate among all major cancer types (1). Pancreatic tumors are genetically complex and resistant to current treatment modalities (4). Progress in the field of molecular treatment based on individual tumor characteristics (personalized/precision oncology) is much needed.

Pigment epithelium-derived factor (PEDF, also known as EPC1 or caspin) was first recognized in retinoblastoma cells, where it induced neuronal differentiation (5). It is a 50-kDa protein encoded by SERPINF1 that belongs to the serpin family (5-7). However, during evolution PEDF has lost its protease-inhibitory activity and has gained other properties, making it part of a subgroup of non-inhibitory serpins (8). PEDF is mainly secreted as a soluble monomeric glycoprotein (7). The pancreas is one of the organs with the most abundant PEDF expression (9). The cell surface receptors that interact with PEDF include PEDFR (also known as ATGL, desnutrin and iPLA2ζ, encoded by PNPLA2), F1 ATPase/synthase, LRP6 and the laminin receptor (7, 10).

The first discovery of the antiangiogenic properties of PEDF was in the eye, where it was found to inhibit vessels from invading the cornea and vitreous body (11). In tumors, PEDF selectively induces apoptosis of endothelial cells in vessels undergoing remodelling (7). PEDF has also been shown to have suppressor-like activity *in vivo* and directly inhibit tumor growth and metastasis in breast cancer (7, 12), while knockdown of PEDF in melanoma cell lines increases the metastatic potential of melanocytes (13). Reduced PEDF levels have also been associated with a worse prognosis in a variety of tumors (7).

In this review, we summarize the role of PEDF in pancreatic cancer. Furthermore, we discuss potential mechanisms of action and regulation of PEDF, with reference to tumor biology and prognosis of pancreatic cancer. Finally, we describe the potential use of PEDF as a novel mode of therapy for pancreatic cancer.

PEDF levels are decreased in pancreatic cancer tissue. Human pancreatic cancer and non-malignant tissue sections have been stained for PEDF by immunohistochemistry.

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Abbrevations: ATP: adenosine triphosphate, EC: endothelial cell, LRP6: low density lipoprotein receptor-related protein 6, NFκB: nuclear factor kappa-light-chain-enhancer of activated B cells, PanIN: Pancreatic Intraepithelial Neoplasia, PEDF: pigment epithelium-derived factor, PEDFR: PEDF-receptor, PPARγ: peroxisome proliferator-activated receptor-γ, TGFβ1, transforming growth factor beta 1, TNM: primary tumor spreading to lymph nodes and metastasizing, VEGF: vascular endothelial growth factor, Wnt: Wingless/Integrated.

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Pancreatic cancer tissues were found to have significantly reduced mean PEDF scores compared to adjacent normal sections (14). Furthermore, only 20% of pancreatic cancer sections demonstrated high PEDF staining, compared to 70% of the non-malignant controls. Approximately 30% of pancreatic cancer tissue had complete loss of PEDF expression, but this was found in less than 5% of adjacent, non-malignant sections (14). PEDF protein expression has also been evaluated in human pancreatic cancer cell lines. Cell lines derived from liver metastases demonstrated a four-fold decrease in PEDF levels compared to cell lines derived from the primary tumor (15). Cell lines derived from ascites displayed an intermediate expression of PEDF compared to the other two groups. These results indicate that induction of a metastatic phenotype could be facilitated by degradation of PEDF and loss of its tumor suppressive properties.

PEDF levels in pancreatic cancer sera. Circulating levels of PEDF have been examined in patients with pancreatic cancer and healthy and benign controls. Serum levels of PEDF were demonstrated to be significantly lower in patients with pancreatic cancer compared to healthy volunteers (16). When compared to benign pancreatic disease, patients with pancreatic cancer were found to have a 75% decrease in PEDF levels with a mean PEDF serum level of 80.5 ng/ml as compared to 344.7 ng/ml for the benign controls (15).

Angiogenesis. Tumors are not able to grow beyond 1-2 mm^3 without recruiting their own blood supply, a process known as 'angiogenic switch' (17). In pancreatic cancer patients, the proangiogenic factor VEGF can increase by approximately 65%, yielding a three-times higher VEGF/PEDF-ratio (15). Interestingly, PEDF has been found to be inversely correlated to microvessel density (MVD) in human pancreatic cancer tissue, with a median MVD at 19.3 in high PEDF expressing tumors, compared to 31.1 in low PEDF expressing tumors (18). In addition, *in vivo* gene transfer studies of PEDF have shown a 70-75% decrease in MVD compared to control tumors in a murine model (16).

Growth and differentiation. PEDF is a key regulator of normal pancreas growth (19). In mice, lack of PEDF leads to increased stromal vascularity and epithelial cell hyperplasia in the pancreas (19). Interestingly, loss of PEDF in a background of mutant *Kras* gene induces pancreatic neoplasia and promotes invasive carcinoma in mice (15). Furthermore, PEDF-deficient mice develop larger and more poorly differentiated lesions compared to control mice (14).

Inflammation. Pancreatic cancer is characterized by a pronounced fibro-inflammatory response (20). Patients with low PEDF expression display increased intratumoral inflammation and fibrosis (14, 21). Loss of PEDF has been found to accelerate pancreatic inflammation *in vivo*. PEDF-deficient mice demonstrate enhanced leukocyte infiltration and increased staining of the pan-myeloid marker CD11b and the macrophage marker F4/80 (14). The mechanisms by which PEDF affects inflammatory-related signaling pathways have been partly defined. PEDF-null mice display increased levels of the pro-inflammatory cytokine IL-8 and decreased levels of the anti-inflammatory cytokine IL-10 (14). Furthermore, PEDF has been found to inhibit macrophage activation *in vitro* through NFκB and ADAM17 (14).

Fibrosis. Enhanced inflammatory responses can lead to increased collagen deposition and fibrosis (22). Pancreatic tumors with loss of PEDF display higher fibrosis scores (14). PEDF-deficient mice are characterized by increased levels of fibrosis and TGFβ1 expression, as well as α-smooth muscle actin expression, corresponding to increased stellate cell activity. Interestingly, treatment of stellate cells with recombinant PEDF reduces collagen IA deposition and endogenous TGFβ1 (14).

Nerves. Neuropathy is a common feature of pancreatic cancer causing severe pain and offering poor prognosis (23). PEDF is a potent neurotrophic factor (24). PEDF promotes the survival and differentiation of a variety of neuronal cells such as retinal neurons (25), glial cells (26), cerebellar granule cells (27, 28), spinal cord motor neurons (29) and neural stem cells (30). It has been suggested that secretion of PEDF by pancreatic cancer cells may lead to sprouting of the nerves toward cancer structures and neural invasion (31, 32). Indeed, a significant association has been found between PEDF expression in pancreatic cancer and neural hypertrophy (31). However, although the nerve caliber in pancreatic cancer is increased, there is a loss of the fine periacinar innervation secondary to the periacinar fibrosis (31).

Adipogenesis. Increased adipocyte density is linked to pancreatic tumor progression (33). Kras/PEDF deficient mice show increased numbers of intrapancreatic adipocytes and peripancreatic fat. The adipocytes in these mice appear enlarged with a higher mean cross-sectional area, as compared to controls. In addition, the expression of the lipid droplet-associated proteins, TIP47 and ADRP, is elevated, while the levels of the lipolytic enzyme PEDFR decrease (15).

Autophagy and Wnt signaling. Autophagy is a cellular recycling mechanism that occurs in the normal pancreatic ductal epithelium and early PanIN lesions and it increases in invasive carcinoma to promote cancer cell survival (34). In biopsies of human PanIN-lesions, PEDF levels inversely correlate to the autophagy marker p62, while PEDF appears to regulate autophagy in PanIN cells through Wnt signaling inhibition and NFκB activation (35).

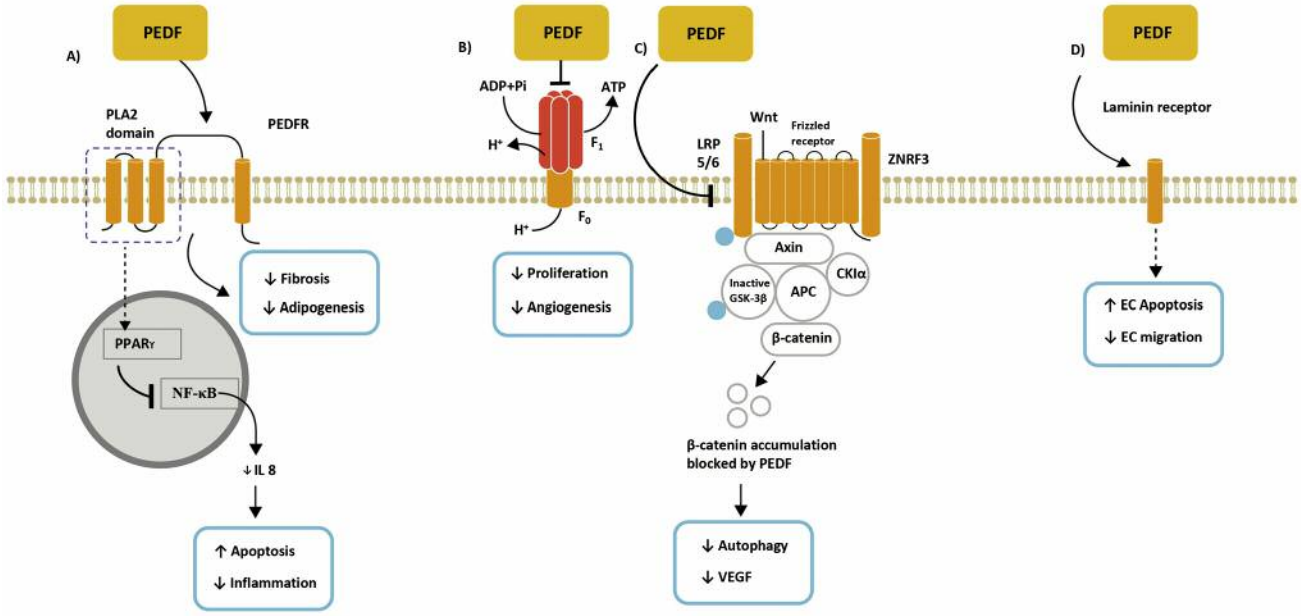

Figure 1. *Proposed pathways of PEDF in pancreatic cancer. A) The PEDF-PEDFR interaction leads to upregulation of PPARγ, which suppresses NFκB and subsequently leads to reduced IL-8 production. The binding of PEDF to its receptor can also reduce pro-fibrotic TGFβ1 expression, as well as hydrolysis of triglycerides. B) PEDF binds to F1 ATP-synthase and inhibits the production of ATP. C) PEDF has been shown to inhibit Wnt signaling by binding to the co-receptor LRP6. This leads to decreased autophagy. D) PEDF can bind to the laminin receptor, which inhibits angiogenesis through apoptosis, as well as the migration of endothelial cells, through as yet unknown pathways (38). The laminin receptor is also present in pancreatic cancer cells (31). Figure adapted from reference (7).*

Metastasis. Most patients with pancreatic cancer are diagnosed with metastatic disease (36). In this scenario, understanding the involvement of PEDF in mechanisms underlying metastatic progression becomes increasingly important. Knockdown of PEDF in pancreatic cell lines can increase cellular motility (15), while PEDF deficient mice are more prone to develop distant metastasis (14, 15). Clinical observations suggest that PEDF expression is inversely correlated to TNM stage and liver metastasis (18).

Patient survival. Correlation between PEDF immuno-reactivity and patient survival has been evaluated in several studies (18, 31). The survival analysis from these studies has revealed a statistically significant correlation between high PEDF expression and prolonged survival of pancreatic cancer patients (18, 31). Importanly, PEDF expression has been identified as an independent prognostic factor in these patients (18).

Therapeutic potential. The therapeutic potential of PEDF has been evaluated in models of lentiviral gene transfer. Introduction of PEDF into human pancreatic cancer cells using this system has resulted in the inhibition of tumor growth in murine models where the gene transfer was performed both subcutaneously and peritoneally (16). Furthermore, intratumoral administration of lentiviral-PEDF in already established tumors can also inhibit tumor growth (16). In addition, lentiviral gene transfer for the overexpression of the known cancer suppressor gene DAPK1 has affected the expression levels in a range of proteins, including the increase of PEDF levels (37).

Conclusion

PEDF is a non-inhibitory member of the serine protease inhibitor gene family, with antitumoral, antiangiogenic and antimetastatic properties in pancreatic cancer (15). PEDF is a ligand for several receptors, and its interaction with these receptors may trigger several pathways that mediate signals between the cancer cells and the tumor microenvironment (7), as shown in Figure 1. Analyses of PEDF levels in serum and tissue samples from patients with pancreatic cancer demonstrate that decreased PEDF levels significantly correlate with stage, distant metastasis and survival (15, 16, 18, 31). The measurement of serum or tumoral tissue levels of PEDF may become an important tool for improving early diagnosis or for offering more appropriate treatment. Further studies regarding the delivery systems and the *in vivo* effect of PEDF in pancreatic cancer are necessary to elucidate the potential of PEDF as a novel therapeutic option.

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