

PDGF-D Signaling: A Novel Target in Cancer Therapy

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Abstract: Platelet-derived growth factor-D (PDGF-D) is a newly recognized growth factor that can regulate many cellular processes, including cell proliferation, transformation, invasion, and angiogenesis by specifically binding to and activating its cognate receptor PDGFR- β . The functions of PDGF-D in human cancer progression are largely unknown. We discuss here the role of PDGF-D signaling pathway in cancer and how its deregulation is involved in tumor development and progression to metastatic disease.

Key Words: PDGF-D, cancer, EMT, signaling, therapy, invasion, stem cells, nutrition.

BACKGROUND OF PDGF-D SIGNALING

Platelet-derived growth factors (PDGFs) regulate diverse cellular functions in cells and tissues, and in normal embryonic development [1-3]. The PDGFs are composed of four different polypeptide chains encoded by different genes. Four PDGF family members have been identified to date: PDGF A-D. The four PDGF chains assemble into disulphide-bonded dimers *via* homo- or hetero-dimerization, and five different dimeric isoforms have been described so far; PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC and PDGF-DD. It is notable that no hetero-dimers involving PDGF-C and PDGF-D chains have been described [4]. The PDGFs have a common structure with the typical growth factor domain involved in the dimerization of the two subunits, and in receptor binding and activation. PDGF-A and PDGF-B have short N-terminal extensions that undergo intracellular proteolytic processing for activation, while both PDGF-C and PDGF-D chains display a distinct protein domain, the so called CUB domain, as part of their N-terminal extensions. Several reports have indicated that the CUB domain of PDGF-D has to be cleaved extra-cellularly to make the COOH-terminal growth factor domain active for PDGF-D binding to its receptor. Those readers who are interested in learning more on the structures of the PDGF are referred to other published reviews [4, 5]. PDGFs exert their cellular effects by activating two structurally related receptor tyrosine kinases, PDGFR- α and PDGFR- β . The PDGF-A, PDGF-B and PDGF-C are secreted as homo-dimers or heterodimers and bind to dimeric PDGF receptors (PDGFR) composed of α - and/or β -chains, while PDGF-D specifically binds to and activate its cognate receptor PDGFR- β [1]. The physiological relevance of the ability of PDGFs to activate PDGFR hetero-dimers is unclear at present.

PDGF-D SIGNALING IN CANCER

The growing body of literature strongly suggests the effects of PDGF signaling in cancer cell growth, invasion and

metastasis. Since the 1970s, PDGF-A and PDGF-B have been extensively studied and well characterized, while PDGF-D was discovered only recently, and the functions of PDGF-D in human cancer progression are largely unknown. It has been reported that PDGF-D signaling is frequently deregulated in human malignancies with up-regulated expression of PDGF-D found in prostate, lung, renal, ovarian, brain, and pancreatic cancer [6-9]. It is well known that PDGF-D interacts with PDGFR- β and activates downstream signaling, such as phosphatidylinositol 3-kinase (PI3K)/Akt, resulting in tumor development and progression. However, the precise role and mechanism of PDGF-D for tumor cell proliferation, invasion, and angiogenesis has not been fully elucidated. Li *et al.* found that PDGF-D is a potent transforming growth factor for NIH/3T3 cells, and the transformed cells displayed stress fiber reorganization, increased proliferation rate, anchorage-independent growth in soft agar, ability to induce tumors in nude mice, and up-regulation of vascular endothelial growth factor. These results suggest that PDGF-D induces cellular transformation and promotes tumor growth by accelerating the proliferation rate of the tumor cells, and by stimulation of tumor neo-vascularization [1]. Although only a handful of published papers document the role of PDGF-D in cancer; however these limited results clearly suggest that PDGF-D plays an important role in the oncogenesis of several malignancies. Here, we summarized the functional role of PDGF-D in different neoplasms.

Prostate Cancer

Prostate cancer is the second leading cause of cancer-related deaths in men in the United States. The progression of prostate cancer is involved in several signaling pathways including PDGF signaling. It has been reported that approximately 80% of prostate tumor tissues express PDGFRs at both primary and metastatic tumors, and inhibition of PDGFR signaling using PDGFR inhibitor Gleevec significantly reduces prostate cancer bone metastasis in a mouse model [10]. Ustach *et al.* found that human prostate carcinoma LNCaP cells are capable of processing full-length PDGF-D into a biologically active PDGF ligand for PDGFR- β activation, and that this processing most likely occurs at or near the cell surface. PDGF-D expression greatly enhances

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prostate carcinoma cell interaction with the surrounding stromal layers in a severe combined immunodeficient (SCID) mouse model, demonstrating a potential oncogenic activity of PDGF-D in human prostate cancer progression [6].

Ustach *et al.* also found that prostate carcinoma cells LNCaP and PC3 could auto-activate latent full-length PDGF-D into its active form under serum-independent conditions, and that this auto-activation is inhibited by PAI-1, an urokinase plasminogen activator (uPA)/tissue plasminogen activator (tPA) inhibitor. Interestingly, uPA, but not the closely related protease tPA, is capable of processing recombinant latent PDGF-DD into the active form. They also identified the uPA cleavage site between the CUB and PDGF domains of the full-length PDGF-D by mutational analysis and showed that PDGF-D and uPA co-localize in human prostate carcinoma. This evidence provides a direct link between uPA and PDGF-D-mediated cell signaling, which may contribute to the progression of prostate cancer [7].

Kong *et al.* have found that prostate cancer PC3 cells transfected with PDGF-D (PC3 PDGF-D cells) exhibit a rapid growth rate and increased invasion *in vitro*, which were associated with a high level of mTOR activity and increased Bcl-2 expression, but reduced activity of Akt. Moreover, condition medium from PDGF-D over-expressing PC3 cells induced tube formation of human umbilical vein endothelial cells (HUVECs) [11], and these results clearly suggest that PDGF-D could induce angiogenesis; hence PDGF-D could be an important target for prostate cancer therapy.

Pancreatic Cancer

Pancreatic cancer is one of the most common cancers and is the fourth leading cause of cancer-related death in the United States. Pancreatic cancer like many other tumors has been shown to over-express the PDGF-D. It has been reported that PDGF-D is highly expressed in human pancreatic adenocarcinoma specimens, in chronic pancreatitis associated with pancreatic adenocarcinoma, and in different human pancreatic cancer cell lines, suggesting that PDGF-D could be important in human pancreatic cancer progression [8]. It was found that down-regulation of PDGF-D in pancreatic cancer cells leads to the inactivation of Notch-1 and NF- κ B DNA-binding activity and, in turn, down-regulates the expression of its target genes, such as *VEGF* and *MMP-9*. Consistent with these results, down-regulation of PDGF-D by small interfering RNA decreased tumor cell invasion, whereas PDGF-D over-expression by cDNA transfection led to increased cell invasion in pancreatic cancer cells. Moreover, conditioned medium from PDGF-D siRNA-transfected cells showed reduced levels of VEGF and, in turn, inhibited the tube formation of HUVECs, suggesting that down-regulation of PDGF-D leads to the inhibition of angiogenesis [8]. These results are consistent with findings reported earlier in prostate cancer [11]; thus PDGF-D appears to be a novel therapeutic target.

Renal Cancer

Renal cancer (renal cell carcinoma; RCC) is the second most lethal of the urological cancers in the United States. Recent data suggest that over-expression of PDGF-D in renal cancer SN12-C cells promoted tumor growth, angiogenesis

and metastasis due to increased expression of angiopoietin-1 and MMP-9 in an orthotopic mouse model [9]. Specifically, Xu *et al.* reported that PDGF-D over-production in SN12-C cells increased the proliferation and migration of cells *in vitro* and improved perivascular cell coverage *in vivo*. Over-expression of PDGF-D led to increased expression of angiopoietin-1 and matrix metalloproteinase-9 in tumor tissues. Inhibition of PDGF-D expression by short hairpin RNA interference (shRNAi) and blockage of PDGFR β signaling by Gleevec inhibited the growth and lung metastasis of SN12-C cells grown orthotopically in SCID mice [9]. These results clearly provide pre-clinical evidence showing that PDGF-D could be a molecular target in RCC.

Glioblastoma

Glioblastoma is the most common form of malignant brain tumor. Glioblastoma is known to contain numerous genetic and physiological alterations affecting cell survival and proliferation. One of the most common alterations is PDGF signaling. Lokker *et al.* demonstrated the expression of PDGF-D and PDGFRs in glioblastomas. They found that PDGF-D was expressed in most of the glioblastoma cell lines and primary glioblastoma multiforme tumors examined, whereas little or no expression was found in normal brain tissue [12]. Blocking PDGF-D/PDGFR signaling inhibited survival and mitogenic pathways in the glioblastoma cell lines and prevented glioma formation in a nude mouse xenograft model [12]. As a strategy for blocking PDGFR signaling, CT52923, a potent selective small molecule piperazinyl quinazoline kinase inhibitor of the PDGFR, was used [12]. In the NIH/3T3 cells, CT52923 blocked PDGF autocrine-mediated phosphorylation of PDGFR, Akt, and mitogen-activated protein kinase (MAPK), while having no effect on *v-fms* or *V12-ras*-mediated Akt or extracellular signal-regulated protein kinase (ERK) phosphorylation. More importantly, oral administration of CT52923 to nude mice caused a significant reduction (61% reduction) in tumor growth of NIH/3T3 cells transformed by PDGF [12]. Thus, these results suggest that targeting PDGF by CT52923 could be therapeutically important for glioblastoma.

Schwannomas

Schwannomas are common tumors of the nervous system occurring sporadically and in patients with the cancer predisposition syndrome neurofibromatosis type 2. Recently, Ammoun *et al.* reported that PDGF-D activated PDGFR β and its downstream genes such as extracellular signal-regulated kinase 1/2 (ERK1/2) in human Schwannoma cells. They showed increased proliferation of schwannoma cells upon stimulation with PDGF-D, whereas no effects were observed in schwann cells. Inhibition of MAPK/ERK kinase 1/2 (MEK1/2) decreased PDGF-D-mediated proliferation to basal levels, suggesting that ERK1/2 pathway is involved in this process [13]. Their data also suggest cooperation between PI3K, PKC, and Src in PDGF-D-mediated ERK1/2 activation. However, Ras is not activated by PDGF-D and is not involved in the PDGFR β -mediated pathway [13]. Interestingly, PDGF-D engages different pathways to activate ERK1/2 that is localized to different intracellular compartments. The p-ERK1/2 pathway at 42/44 kDa (cytosol) favors a model in which c-Raf is activated by Src and PKC,

where the later is activated by PI3K (via PDK1), which directly binds to phosphorylated/activated PDGFR β . These results suggest that a combined therapy targeting different pathways including PDGF-D pathway might be appropriate for treating schwannoma.

Melanoma and other Cancers

Furuhashi *et al.* demonstrated that PDGF-D stimulation of pericytes in the B16 melanoma mouse tumor model leads to an increased pericyte abundance in tumor vessels and to an increase in tumor growth rate, which occurs in the absence of increase in vessel density. Specifically, the volumes of tumors derived from PDGF-D transfected B16 cells were significantly increased as compared with tumors from control transfected cells [14]. PDGF-D signaling is also frequently found to be over-expressed in other malignancies in humans. Yang *et al.* reported that PDGF-D is up-regulated in gastric cancer patient tissue samples compared with normal tissue [15]. However, the exact mechanisms of PDGF-D function in other cancer need further in-depth investigation.

PDGF-D AND CANCER STEM-LIKE CELLS

It has been suggested that the capability of a tumor to grow and propagate is likely due to a small subset of cells within the tumor, termed cancer stem-like cells (CSCs). Although the concept of "cancer stem-like cell" was first proposed more than 150 years ago, it has become more attractive recently due to advances in stem cell biology, leading to the identification of these cells from a wide variety of human cancers [16]. CSCs have been identified and isolated from tumors of the hematopoietic system, breast, lung, prostate, colon, brain, head and neck, and pancreas [17]. CSCs are able to self-renew, differentiate, and regenerate to a phenotypic cells of the original tumor when implanted into the severe combined immunodeficient mouse [17]. The concept of CSCs have generated considerable attention in recent years, which is likely to provide clear understanding of tumor biology, and for designing novel therapy targeted toward these cells for the complete eradication of tumor growth. It has been reported that altered PDGF signaling affects the function of a variety of mammalian stem cells. PDGFR α signaling occurs early in the adult neuronal stem cell lineage and may help regulate the balance between oligodendrocyte and neuron production. Excessive PDGF activation in the subventricular zone (SVZ) in the niches where stem cells are usually located is sufficient to induce changes that are typical "hallmarks" associated with early stages of tumor formation.

Recently, Kong *et al.* reported that platelet derived growth factor (PDGF) signaling contributes to epithelial-mesenchymal transition (EMT) phenotype that is known to be reminiscent of "cancer stem-like cells" which, in turn, regulates cancer cell invasion and angiogenesis [18]. The authors have found that PC3 PDGF-D cells (PC3 prostate cancer cells stably transfected with PDGF-D cDNA) resulted in a significant induction of EMT as shown by changes in cellular morphology concomitant with the loss of E-cadherin and zonula occludens-1 (ZO-1), and gain of vimentin. They also found activation of mTOR and NF- κ B as well as Bcl-2 over-expression in PC3 PDGF-D cells, which was associated with

enhanced adhesive and invasive behaviors. More importantly, PDGF-D over-expressing PC3 cells (PC3 PDGF-D cells) showed tumor growth in SCID mice much more rapidly than PC3 cells. These results provided a novel mechanism by which PDGF-D promotes EMT, which in turn increased tumor growth, and these results further suggest that PDGF-D could be a novel therapeutic target for the prevention and/or treatment of prostate cancer [18]. Taken together, these results suggested that PDGF-D pathway plays an important role in cancer progression as illustrated in Fig. (1).

PDGF-D AS A CANCER THERAPEUTIC TARGET

We and other investigators have demonstrated that increased expression of PDGF-D and its receptor is detected in many human cancer cells and tissues such as pancreas, brain, lung, prostate, renal cancer [6-9, 18, 19]. PDGF-D displays an oncogenic activity specifically through binding to and activating its cognate receptor PDGFR- β . These results clearly suggest that inactivation of PDGF-D/PDGFR signaling by novel approaches is likely to have a significant impact in cancer therapy. Several small molecule tyrosine kinase inhibitors that block the PDGF receptor are among the many recently developed targeted anticancer therapeutic agents. For example, Lokker *et al.* found that treatment with the PDGFR antagonist CT52923 inhibited survival and/or mitogenic pathways in the glioblastoma cell lines and prevented glioma formation in a nude mouse xenograft model [12]. In addition, imatinib (STI571 or Gleevec), which is a selective tyrosine kinase inhibitor and particularly of PDGF-R and c-Kit, exhibited encouraging results with respect to its inhibitory effect in cell growth and invasion potential in human breast cancer cell lines [20]. Inhibiting phosphorylation of PDGFR by treatment with the imatinib and the chemotherapeutic agent paclitaxel reduces the incidence and size of human prostate cancer bone lesions in nude mice [21]. Interestingly, tumor-associated endothelial cells, rather than tumor cells themselves, appear to be the target of imatinib in prostate cancer bone metastasis [21]. Moreover, the combination of imatinib with gemcitabine led to a further tumor growth inhibition and improved mice survival by decreasing rate of tumor cell proliferation and by increasing the number of apoptotic tumor cells in malignant mesothelioma, suggesting that imatinib enhances the therapeutic response to gemcitabine [19]. Administration of the PDGF-D neutralizing, fully humanized monoclonal antibody CR002, in the acute phase of progressive anti-Thy 1.1 glomerulonephritis, reduced glomerular and secondary tubulointerstitial damage [22]. The phase-I study of CR200 has been conducted, which showed that CR002 was safe and well-tolerated at different doses from 0.3 to 30 mg/kg tested as a single i.v. administration. Moreover, CR002 had a long half-life, low clearance but limited tissue distribution [23].

To our knowledge, there is no report regarding the small chemical inhibitors of PDGF-D. However, we have found that B-DIM (a well known chemopreventive agent) significantly inhibited the expression and activation of PDGF-D in PC3 PDGF-D cells. B-DIM also inhibited both mTOR and Akt in PC3 PDGF-D cells, which were correlated with decreased cell proliferation and invasion [11]. Moreover, conditioned medium from PC3 PDGF-D cells significantly increased the tube formation (an indirect measure of angio-

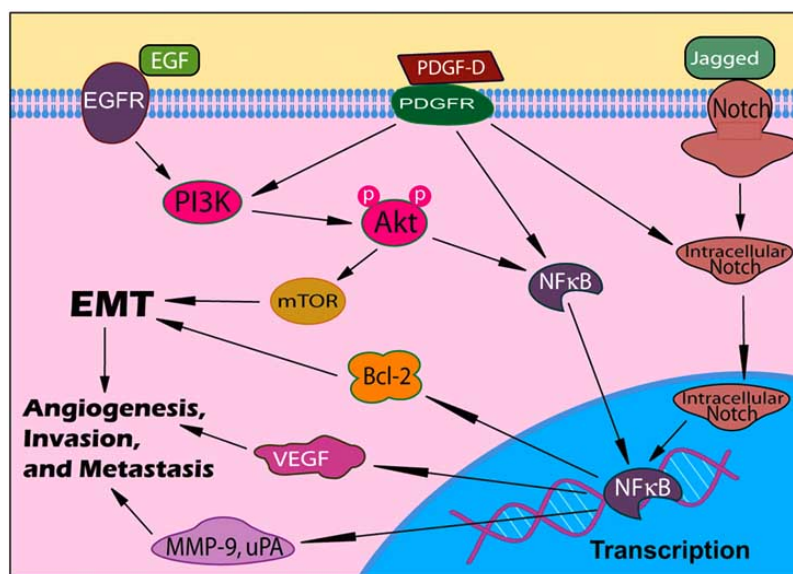


Fig. (1). A mechanistic diagram showing how PDGF-D could promote cancer progression.

genesis) of human umbilical vein endothelial cells (HU-VECs), which was inhibited by B-DIM treatment, resulting in the concomitant reduction of full-length and active form of PDGF-D. These results suggest that B-DIM could serve as a novel and efficient chemopreventive and/or therapeutic agent by inactivation of both mTOR and Akt activity in PDGF-D over-expressing prostate cancer cells [11]. These findings also suggest that PDGF-D down-regulation, especially by B-DIM (non-toxic agents from dietary sources) could be a novel therapeutic approach for the treatment of human cancers by targeting inactivation of PDGF-D signaling as well as by inhibiting angiogenesis. However, further in-depth studies including mechanistic *in vitro* studies, *in vivo* animal experiments and clinical trials are needed to fully appreciate the consequence of the down-regulation of PDGF-D signaling by non-toxic dietary chemopreventive agents. As such, this article could stimulate further research in this field for the development of non-toxic approaches for cancer therapy by targeting PDGF-D signaling.

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