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Emerging roles of PDGF-D in EMT progression during tumorigenesis

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Abstract

Platelet-derived growth factor-D (PDGF-D) signaling pathway has been reported to be involved in regulating various cellular processes, such as cell growth, apoptotic cell death, migration, invasion, angiogenesis and metastasis. Recently, multiple studies have shown that PDGF-D plays a critical role in governing Epithelial-to-Mesenchymal Transition (EMT), although the underlying mechanism of PDGF-D-mediated acquisition of EMT is largely unclear. Therefore, this mini review will discuss recent advances in our understanding of the role of PDGF-D in the acquisition of EMT during tumorigenesis. Furthermore, we will summarize the function of chemical inhibitors and natural compounds that are known to inactivate PDGF-D signaling pathway, which leads to the reversal of EMT. In summary, inactivation of PDGF-D could be a novel strategy for achieving better treatment outcome of patients inflicted with cancers.

Keywords

PDGF-D; EMT; migration; invasion; miRNA; natural compounds; stem cells

Introduction

Cancer is a major public health problem and one of the most common causes of deaths in the world. It has been estimated that 1,638,910 new cancer cases will be diagnosed and 577,190 cancer-related deaths will occur worldwide in 2012¹. Currently, 33% women and 50% men

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Conflict of Interest

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will develop cancer in their lifetime in the United States ². Although progress has been made in reducing cancer incidence, the number of individuals diagnosed with cancer each year is still increasing which is in part due to aging and population growth in some countries ². Moreover, five-year relative survival rate is approximately 65% for all human cancers. One of the reasons for this high mortality rate is due to the lack of understanding of the exact mechanism underlying cancer development and progression, and thus resulting in the shortage of effective treatment strategies ¹. Therefore, it is pivotal to explore the molecular mechanisms how cancer is developed and discover novel drugs for the treatment of human cancer patients.

In recent years, although the causes of carcinogenesis are largely unknown, studies indicated that many factors and co-factors such as family history, smoking, obesity, and diet are correlated with increased incidence of cancer ^{3,4}. Furthermore, it has been demonstrated that many genes and cellular signaling pathways such as Notch ⁵⁻⁷, PI3K (phosphatidylinositol 3-Kinase)/Akt ^{8,9}, Hedgehog ^{10,11}, Wnt ^{5,12}, NF- κ B (nuclear factor-kappa B) ¹³⁻¹⁵ have been found to play critical roles in human tumorigenesis. Among many of these signaling pathways, PDGF-D has recently gained tremendous amount of attention due to its involvement in carcinogenesis, which is mediated through regulating many cellular functions ¹⁶⁻²¹. More importantly, PDGF-D has been found to regulate the process of Epithelial-to-Mesenchymal Transition (EMT) that is important for tumor metastasis ²²⁻²⁷. Therefore, in this review article, we will briefly introduce the role of PDGF-D signaling pathway and further discuss its role in the acquisition of EMT phenotype. Lastly, we will describe potential application of PDGF-D inhibitors for the prevention and/or treatment of human cancers in the near future.

PDGF-D signaling pathway

PDGF-D belongs to PDGF family that is comprised of four polypeptide chains encoded by four genes, known as PDGF-A, B, C, and D ²⁸. It is clear that in order to exert its function, PDGF-D needs to be assembled into dimers via homodimerization and also requires to activate its full-length PDGF-D into a biologically active PDGF-D form ²⁸. It is worth mentioning that PDGF-D exerts its cellular effects through specifically binding to and activating its cognate receptor PDGFR- β , resulting in phosphorylation of PDGFR- β and subsequent triggering of a number of downstream signaling pathways ²⁹. In recent years, studies have shown that multiple cellular signaling pathways such as matriptase, PTEN (phosphatase and tensin homologue deleted on chromosome 10), ROS (reactive oxygen species), and uPA (urokinase-type plasminogen activator) govern the PDGF-D activity ^{16,18,19,30-33}. Recently, we found that activated K-ras and INK4a/Arf deficiency led to high expression of PDGF-D pathway ²⁷. On the other hand, PDGF-D could regulate various cellular pathways including PI3K/Akt, NF- κ B, Notch, ERK (extracellular-regulated kinase), mTOR (mammalian target of rapamycin), MAPK (mitogen-activated protein kinase), VEGF (vascular endothelial growth factor), MMPs (matrix metalloproteinases), Cyclin D1, and Bcl-2 to facilitate its oncogenic functions ^{22-24,34-37}.

In agreement with the oncogenic function of PDGF-D in human malignancies, over-expression of PDGF-D has been observed in a variety of cancers including prostate, lung, renal, ovarian, brain, and pancreatic cancer ^{38,39}. For example, we found that PDGF-D is highly expressed in pancreatic adenocarcinoma specimens as well as in chronic pancreatitis associated with pancreatic adenocarcinoma ³⁶. In line with this notion, we also observed over-expression of PDGF-D in breast cancer tissues ²⁶. Similarly, PDGF-D is upregulated in primary prostate cancer and bone metastases ^{16,18,30}. To further support the role of PDGF-D in tumorigenesis, Yang et al. reported that PDGF-D is overexpressed in gastric cancer patient tissue samples compared with normal tissues ⁴⁰. Moreover, PDGF-D was found to be

elevated in the sera of ovarian, renal, lung and brain cancer patients, indicating that PDGF-D plays a crucial role in certain human cancers ⁴¹. Because several excellent published reviews have discussed the roles of PDGF-D in cell growth, apoptosis, and angiogenesis ^{28, 29, 38, 39, 42, 43}, in the following paragraph we will describe the novel functions of PDGF-D in regulating EMT in human cancer, which have recently burst onto the scene.

The Epithelial-to-Mesenchymal Transition (EMT)

It is known that EMT is essential for numerous developmental processes during embryogenesis ⁴⁴. Morphologically, epithelial cells lose their apical-basal polarization and adopt front-to-back polarization. Moreover, cells acquire mesenchymal characteristics such as increased invasive and motility characteristics ⁴⁴. After completion of embryogenesis, it has been believed that EMT is switched off and put to rest. In specific physiological condition such as wound healing, EMT might be reactivated ⁴⁴. Various molecular markers have been used to distinguish mesenchymal cells from epithelial cells which have helped in the identification of the EMT phenotypic cells ^{45, 46}. E-cadherin, the best-characterized member of epithelial markers, is lost upon EMT, leading to migration ⁴⁷. The mesenchymal markers such as Snail, Slug, ZEB1, and Twist have been found to repress the expression of E-cadherin. Vimentin and fibronectin as mesenchymal molecules provide a flexible structure and promote cells to move ^{44, 47}.

Recently, accumulating evidence suggests that cancer cells reactivate the processes of EMT in order to migrate to distant tissue sites ⁴⁴. Due to increased cell migratory capacity after EMT, EMT is believed to be involved in tumor metastasis ⁴⁴. So far, many growth factors and cytokines as well as cellular signaling pathways have been found to trigger EMT program such as TGF- β (transforming growth factor beta), Wnt, FoxM1 (forkhead box protein M1), NF- κ B, EGF (epidermal growth factor), HGF (hepatocyte growth factor) and Notch ^{44, 47–49}. For example, we found that over-expression of FoxM1 in pancreatic cancer cells induced EMT ⁵⁰. In addition, we also revealed that up-regulation of sonic hedgehog contributes to TGF- β -induced EMT in NSCLC cells ⁵¹. Recently, the growing body of literature strongly suggests that PDGF-D plays a critical role in governing EMT ^{22–27}. Therefore, we will highlight the function of PDGF-D in triggering EMT in a variety of human cancers.

The role of PDGF-D in the processes of EMT

Several lines of evidence have revealed that PDGF-D could facilitate EMT. The group led by Dr. Sarkar demonstrated that overexpression of PDGF-D in prostate cancer cells caused changes in the morphology with elongated and irregular fibroblastoid appearance ²⁴. Consistent with these changes, PDGF-D-overexpressing cells showed loss or relocation of E-cadherin at cell-cell junction, and disrupted ZO-1 (zonular occludens-1) in tight junction ²⁴. Concomitantly, β -catenin was relocated from cell membrane to the nuclear compartment by PDGF-D overexpression ²⁴. In addition, increased expression levels of vimentin and nestin were also observed in PDGF-D-overexpressing cells ²⁴. Recently, we found that PDGF-D knockdown in Rink cells led to down-regulation of mesenchymal marker vimentin ²⁷. Taken together, PDGF-D overexpression contributes to EMT in human cancers.

Although the molecular mechanisms by which PDGF-D triggers EMT are largely unknown, multiple studies have indicated that PDGF-D-mediated EMT could be mediated through several cellular signaling pathways such as mTOR, Notch, NF- κ B, CXCR4 (C-X-C chemokine receptor type 4), and Bcl-2 ^{22–27}. Strikingly, microRNAs (miRNAs) have been reported to regulate PDGF-D-mediated EMT ²³. Furthermore, PDGF-D-induced EMT

phenotypic cells have cancer stem-like cell characteristic²². These findings provide compelling evidence for the role of PDGF-D in regulating EMT via multiple mechanisms.

PDGF-D-mediated EMT is mediated through multiple signaling pathways

mTOR signaling pathway

mTOR, an evolutionarily conserved Serine/Threonine kinase, belongs to the PI3K-related protein kinase subfamily⁵². It is known that mTOR regulates multiple cellular processes including cell proliferation, apoptotic cell death, cell metabolism and autophagy⁵³. To exert its functions, mTOR assembles into two sub-complexes, known as mTORC1 (mTOR complex 1) and mTORC2 (mTOR complex 2). mTORC1 is comprised of five components, such as DEPTOR (DEP-domain-containing mTOR-interacting protein), mTOR, mLST8/GβL (mammalian lethal with Sec13 protein 8/G protein β-subunit-like protein), PRAS40 (proline-rich Akt substrate of 40 kilodaltons), and Raptor (regulatory-associated protein of mTOR)⁵³. mTORC2 consists of six components, DEPTOR, MAPKAP1 (mitogen-activated protein kinase associated protein 1; also known as mSIN1), mLST8/GβL, mTOR, PROTOR (protein observed with Rictor-1)/PRR5 (proline-rich protein 5) and Rictor (Rapamycin-insensitive companion of mTOR)⁵⁴.

It has been well documented that deregulation of the mTOR pathway occurs in many human cancers⁵⁵. Moreover, mTORC1 facilitates its oncogenic function through regulating its downstream targets such as S6K (p70 S6 ribosomal kinase) and 4E-BP1 (the eukaryotic translation initiation factor 4E-binding protein 1), while mTORC2 phosphorylates Akt and SGK1 (serum/glucocorticoid regulated kinase 1) to activate their kinase activation⁵⁶. Furthermore, mTORC2 mediates the actin cytoskeletal organization, leading to increased tumor cell invasion and metastasis⁵⁴. Consistent with this notion, studies from different groups have suggested that mTOR signaling is associated with cancer progression and metastasis⁵⁶. Accordingly, mTOR signaling has been found to be significantly elevated in a variety of human cancers. Moreover, inhibiting mTOR signaling by its inhibitor rapamycin attenuated tumor cell migration and invasion⁵⁷.

mTOR has been reported to drive cancer-associated EMT^{58, 59}. For example, inhibition of mTORC1 and mTORC2 induced the reversal of EMT to MET (mesenchymal-to-epithelial transition) with increased cell-cell contact and decreased actin cytoskeletal remodeling, suggesting that mTORC1 and mTORC2 could regulate EMT in human cancers⁵⁸. In supporting the role of mTOR signaling in the regulation of EMT, it has been observed that TGF-β-induced EMT is mediated through induction of mTORC2 kinase activity, demonstrating that mTORC2 is an essential downstream molecule of TGF-β signaling, and represents a novel target for the inhibition of EMT⁵⁹. Recently, our studies have shown that over-expression of PDGF-D in prostate cancer cells promoted cell proliferation and tumor growth *in vitro* and *in vivo* through activation of mTOR downstream targets S6K and 4E-BP1²⁴. More importantly, we found that activation of mTOR pathway is involved in EMT induced by PDGF-D²⁴. To support this concept, we observed an increased activation of mTOR concomitant with loss of E-cadherin and gain of increased vimentin and nestin in PDGF-D-overexpressing cells²⁴. To further confirm this notion, knockdown of mTOR increased the expression of E-cadherin and reduced the level of vimentin in PDGF-D-overexpressing cells²⁴. These results provided the direct evidence to support that the activation of mTOR is associated with the acquisition of EMT induced by PDGF-D.

Notch signaling pathway

The Notch signaling pathway is known as a conserved ligand–receptor pathway, which is involved in various cellular processes such as cell proliferation, differentiation, apoptosis, migration, invasion and metastasis⁶⁰. So far, four Notch receptors (Notch-1, Notch-2,

Notch-3, and Notch-4) and five ligands (Dll-1, Dll-3, Dll-4, Jagged-1 and Jagged-2) have been identified⁶⁰. The activation of Notch signaling is triggered after Notch ligand binds to its receptor, thus leading to Notch proteolytic cleavage and releasing the active Notch intracellular domain (NICD). The NICD then translocates into the nucleus and activates its target genes⁶¹. It has been known that Notch downstream targets include the Hes-1 (Hairy enhance of split-1), COX-2 (cyclooxygenase-2), VEGF, MMP-9 (matrix metalloproteinase-9), ERK, Akt, Cyclin D1, c-Myc, p21, p27, p53, etc⁶².

Notch signaling network is frequently deregulated in human malignancies^{6, 63–65}. Recently, Notch signaling pathway has been observed to induce EMT⁶⁶. For example, we showed that forced over-expression of Notch-1 led to the induction of EMT by activation of mesenchymal cell markers in pancreatic cancer cells⁶⁷. In addition, our study demonstrated that over-expression of PDGF-D caused up-regulation of Notch-1 and Jagged-1 in breast cancer cells³⁴. Consistent with this notion, we found that down-regulation of PDGF-D inhibited Notch-1 expression whereas up-regulation of PDGF-D increased Notch-1 activation in pancreatic cancer cells³⁶. Furthermore, PDGF-D overexpression increased the expression of mesenchymal markers vimentin and ZEB2 and decreased the expression of epithelial marker E-cadherin in breast cancer cells³⁴. Taken together, since Notch-1 plays a critical role in regulating EMT, PDGF-D-mediated EMT could indeed be mediated through up-regulation of Notch-1 signaling pathway.

NF- κ B signaling pathway

It has been well documented that NF- κ B plays pivotal roles in multiple cellular processes such as cell growth, cellular apoptotic death, inflammation, differentiation, and stress response¹³. NF- κ B is sequestered in the cytoplasm via association with its inhibitory protein I κ B. Under stimulation conditions, IKK α (I κ B kinase α) and IKK β (I κ B kinase β) phosphorylate I κ B, leading to the degradation of I κ B by E3 ubiquitin ligase, subsequently allowing the NF- κ B to translocate into the nucleus⁶⁸. Then, NF- κ B binds to the DNA and recruits additional transcription factors and regulates the expression of its target genes such as Bcl-2, MMP-9, Cyclin D1 and VEGF^{14, 69}.

NF- κ B has been found to be involved in the development and progression of human cancers¹³. Moreover, NF- κ B was recently found to regulate EMT in cancers⁷⁰. Our studies have demonstrated that PDGF-D controlled NF- κ B activity in breast cancer, prostate cancer, and pancreatic cancer cell lines^{24, 34, 36}. Specifically, over-expression of PDGF-D enhanced NF- κ B expression and its nuclear abundance as well as its DNA-binding activity, leading to the activation of its target genes including VEGF and MMP-9^{34, 36}. On the contrary, down-regulation of PDGF-D led to the inactivation of NF- κ B DNA-binding activity and, in turn, down-regulated the expression of its target genes, such as VEGF and MMP-9^{34, 36}. Since both PDGF-D and its downstream gene NF- κ B induced EMT, it is possible that PDGF-D governs EMT through NF- κ B pathway. It is worth mentioning that NF- κ B could be a Notch-1 target gene in various cancer cells⁷¹. Therefore, we assumed that PDGF-D triggers EMT partly mediated through the regulation of Notch/NF- κ B pathways. However, further in-depth investigation is required to validate this hypothesis in the future.

Bcl-2 signaling pathway

It has been well accepted that Bcl-2 protein family plays a critical role in the apoptotic signaling pathway⁷². The Bcl-2 family of proteins is classified into anti-apoptotic group and pro-apoptotic group⁷². The anti-apoptotic members have Bcl-2, Bcl-xL, Bcl-w and Mcl-1, while the pro-apoptotic Bcl-2 members include Bax, Bak, and BH3-only proteins such as Bim^{73, 74}. Recently, the pro-apoptotic protein Bcl-2 has attracted increasing attention due to its oncogenic functions in addition to being a pivotal regulator of apoptosis. For example,

Choi et al. reported that Bcl-2 promotes invasion and lung metastasis via induction of MMP-2⁷⁵. Similarly, another study from different group showed that Bcl-2 over-expression enhanced *in vitro* invasion and *in vivo* tumor growth through regulating several MMPs such as MMP-2, MMP-7, MT1-MMP, and TIMP1 (tissue inhibitors of metalloproteases-1) and TIMP-2 in melanoma⁷⁶. Furthermore, it has been found that under hypoxia, Bcl-2 protein promotes HIF-1 (hypoxia-inducible factor-1)/VEGF-mediated tumor angiogenesis⁷⁷. Moreover, Bcl-2 regulates HIF-1 α protein stabilization involving the molecular chaperone Hsp90 in hypoxic melanoma cells⁷⁸.

Recently, Bcl-2 was found to be involved in EMT. For example, Wang et al. reported that Bcl-2 plays a critical role in oestrogen-mediated EMT⁷⁹. Specifically, oestrogen signaling inhibits EMT through repressing Bcl-2 expression⁷⁹. To further support the role of Bcl-2 in EMT, one study has shown that Bcl-2 could bind to the EMT-regulating transcription factor Twist1 *in vitro* and *in vivo*, and facilitate the nuclear transport of Twist, leading to transcriptional activation of multiple genes that can promote tumor cell metastasis⁸⁰. This finding indicates a new function of Bcl-2 in the induction of EMT⁸⁰. Consistent with this observation, we demonstrated that over-expression of Bcl-2 in prostate cancer cells decreased the expression of E-cadherin²⁴. Moreover, we revealed that Bcl-2 contributes to EMT induced by PDGF-D overexpression²⁴. First, up-regulation of Bcl-2 was observed in PDGF-D-overexpressing cells with EMT phenotype²⁴. Second, knockdown of Bcl-2 in PDGF-D-overexpressing cells decreased the expression of vimentin, which is the key marker of EMT cells²⁴. Taken together, Bcl-2 could partly contribute to EMT induced by PDGF-D over-expression. It is important to note that Bcl-2 is one of many targets of NF- κ B. Therefore, it is possible that PDGF-D controls EMT partly through the regulation of NF- κ B/Bcl-2 pathways.

CXCR4 signaling pathway

CXCR4 is a G-protein-coupled receptor for SDF-1/CXCL12 (stromal cell-derived factor-1/CXC chemokine ligand 12) that is a small pro-inflammatory chemoattractant cytokine^{81, 82}. SDF-1 binds to CXCR4 and subsequently activates several downstream signaling pathways such as ERK2, protein kinase B, PI3K, MAPK and NF- κ B, which in turn govern cell proliferation, survival and chemotaxis⁸³. In line with the notion that CXCR4 positively regulates multiple oncogenic pathways, over-expression of CXCR4 has been reported in various human cancers including prostate, breast and pancreatic cancer⁸³. Moreover, activation of CXCR4 induced cancer cell migration and adhesion to stromal cells, thus activated growth factors to the tumor cells. Accordingly, inactivation of SDF-1/CXCR4 signaling blocked cancer cell invasion and migration⁸⁴.

CXCR4 has been found to play a critical role in EMT⁸⁴. SDF-1/CXCR4 induced EMT processes, which was in part mediated through the activation of hedgehog signaling pathway in pancreatic cancer cells. This EMT process was inhibited by Smoothened inhibitor cyclopamine, and knockdown of hedgehog targeted Gli-1⁸⁴. Recently, PDGF-D has been found to induce CXCR4 expression in breast cancer cells⁸⁵. Specifically, over-expression of PDGF-D promotes tumor growth and lymph node metastasis which was partly mediated through up-regulation of CXCR4 expression⁸⁵. In support of this, inhibition of CXCR4 abolished PDGF-D-mediated lymph node metastasis⁸⁵. Taken together, these results suggest that the SDF-1/CXCR4 axis is involved in PDGF-D-induced EMT, suggesting that modulating SDF-1/CXCR4 may have clinical applications in the inhibition of EMT and metastatic potential of cancer cells.

miRNA regulates PDGF-D-mediated EMT

It became clear that miRNAs are small regulatory non-coding RNA molecules that negatively regulate the expression of the target genes through interacting with the 3' untranslated regions (UTRs) of specific mRNAs⁸⁶. Therefore, miRNA represses gene expression at a post-transcriptional level by blocking mRNA translation or promoting their degradation⁸⁷. Notably, miRNAs have been found to be involved in the development and progression of human cancers. Without a doubt, miRNAs can serve as tumor suppressor genes or oncogenes depending on the function of their targets⁸⁸. It has been established that miRNAs are involved in cancer pathogenesis including cell growth, migration, invasion, metastasis, and drug resistance⁸⁹.

Increasing evidence indicates that miRNAs play important roles in EMT. For example, miR-150 targets the EMT inducer ZEB1 in esophageal squamous cell carcinoma, leading to the induction of MET-like changes with up-regulation of E-cadherin and down-regulation of vimentin⁹⁰. Han et al. reported that miR-21 regulates EMT via over-expression of HIF-1 α ⁹¹, while inhibition of miR-21 reverses EMT phenotype through inactivation of Akt/ERK by targeting PTEN⁹². Cao et al. found that miR-23 regulates TGF- β -induced EMT by targeting E-cadherin in lung cancer cells⁹³. Recently, we found that miR-200 regulates PDGF-D-mediated EMT in prostate cancer cells²³. Specifically, miR-200 contributes to the regulation of EMT partly through down-regulation of ZEB1, ZEB2 as well as Snail2, and up-regulation of epithelial marker genes such as E-cadherin, stratifin, EpCAM, and connexin 26²³. Re-expression of miR-200b reversed EMT to MET in PDGF-D-overexpressing prostate cancer cells, leading to inhibition of cell adhesion and invasion²³. More importantly, purified PDGF-D protein treatment led to the repression of miR-200 family and increased expression of ZEB2, Snail2 as well as down-regulation of E-cadherin²³. Taken together, these results suggest that miR-200 governs PDGF-D-mediated EMT in human cancers.

PDGF-D-induced EMT-type cells show cancer stem cell features

Accumulating evidence suggests that EMT-type cells have CSCs (cancer stem cells) characteristics in various human malignancies^{94,95}. It is believed that CSCs possess self-renewal activity and generate various cell populations⁹⁶. More importantly, CSCs have been identified and isolated using different sets of stem cell surface markers in a wide variety of human cancers including breast, lung, prostate, colon, brain, head and neck, and pancreatic cancers⁹⁷. Recently, the positive relationship between EMT and CSCs has been observed in human cancers^{48,98}. To support this concept, studies have shown that epithelial cancer stem cells express a wide array of mesenchymal markers. On the other hand, the activation of EMT program has been associated with the acquisition of stem cell traits⁹⁹. For example, Mani et al. reported that the EMT transition generates cells with properties of stem cells in breast cancer⁹⁶. Additionally, this group showed that normal and neoplastic breast stem-like cells express mesenchymal markers⁹⁶. At the same time, another group confirmed that generation of breast cancer stem cells is mediated through EMT induction by the activation of the Ras-MAPK pathway¹⁰⁰. Similarly, Shah et al. revealed that EMT-type pancreatic cancer cells have up-regulation of the stem cell markers CD24, CD44, and ESA¹⁰¹. Consistent with this finding, our studies have demonstrated that pancreatic cancer cells with EMT phenotype showed increased sphere-forming capacity and overexpression of CSC surface markers^{50,67}. Therefore, the connection between EMT and CSCs demonstrated that the EMT process is involved in tumorigenesis through its cellular traits associated with motility, invasiveness and metastasis, as well as its stem cell traits with self-renewal capacity.

Although EMT is associated with CSCs, the mechanism by which EMT-type cells acquire CSCs features remains largely elusive. Recently, PDGF-D was found to play a critical role in governing EMT that also showed connection with CSCs²². In concordance with this notion, we observed that prostate cancer cells with EMT phenotype induced by PDGF-D displayed CSCs features including up-regulation of Sox2, Nanog, Oct4, Lin28B and Notch-1, which is consistent with enhanced sphere-forming ability as well as rapid tumor growth *in vivo*²². Moreover, reversal of EMT decreased prostasphere-forming ability of EMT-type cells and down-regulated the expression of Lin28B and Notch-1, leading to the repression of self-renewal capability²². Recently, we found that activated K-Ras and INK4a/Arf deficiency promote aggressiveness of pancreatic cancer by the acquisition of EMT phenotype and induction of CSC characteristics partly through the activation of PDGF-D²⁷. Specifically, the deletion of Ink4a/Arf in K-Ras (G12D) expressing mice showed high expression of PDGF-D in the pancreatic tumor and tumor-derived cell line (Rink-1 cells)²⁷. Depletion of PDGF-D in Rink-1 cells led to reduced pancreatosphere formation and decreased expression of EZH2, CD44, EPCAM, and vimentin, indicating that PDGF-D plays a critical role in the induction of EMT consistent with CSCs phenotype²⁷. More recently, it has also been reported that tissue-resident stem cells induce EMT through interaction with cancer microenvironment via PDGF-D, thereby leading to increased numbers of CSCs and promoting tumor growth²⁵. However, further investigation is required to investigate how PDGF-D-induced EMT-type cells acquire CSCs characteristics in human cancers.

PDGF-D as a cancer therapeutic target

Since PDGF-D plays oncogenic activity through the regulation of tumor cell growth, invasion, and metastasis that are associated with EMT and CSCs, targeting PDGF-D signaling pathway could be a novel strategy for the treatment of human cancers. However, to our knowledge, small chemical inhibitors of PDGF-D have not been developed so far with proven anti-tumor efficacy in humans. Because PDGF-D exerts its function via activation of its receptor PDGFR- β , multiple small molecule tyrosine kinase inhibitors that inactivate PDGFR have been reported^{102–105}. For example, imatinib, a tyrosine kinase inhibitor that interferes with the phosphorylation and activation of PDGFR β , has been reported to inhibit cell growth and invasion in various cancer cell lines¹⁰⁶. Sunitinib, a multi-tyrosine kinase inhibitor, reduces survival and migration of human meningioma cells by targeting PDGFR¹⁰⁷. Moreover, Sunitinib could serve as radiosensitizer for human prostate cancer partly through the inhibition of PDGFR and VEGFR¹⁰². ABT-348 exerts its anti-tumor activity via targeting the VEGFR, PDGFR, Aurora, and SRC kinase¹⁰³. AL3810 exhibits potential anti-angiogenic and anti-tumor activity partly through the inhibition of PDGFR in human cancer cells¹⁰⁵. Cediranib was also found to reduce intraosseous growth of prostate tumor and associated bone reaction through the suppression of PDGFR and VEGFR¹⁰⁸. TKI258, a tyrosine kinase inhibitor of FGFR, PDGFR, and VEGFR, has also been reported to inhibit tumor growth, angiogenesis, and metastasis in pancreatic cancer¹⁰⁴. Tricin, a naturally occurring flavone, exhibited anti-oncogenic activity through blocking tyrosine phosphorylation of PDGFR β and its downstream signaling molecules ERK1/2¹⁰⁹. It is important to note that most small molecule tyrosine kinase inhibitors target multiple tyrosine kinases.

Although there is no specific inhibitor for PDGF-D, a fully-humanized monoclonal antibody against PDGF-D, known as CR002, has been developed and shown to be safe in a phase I study¹¹⁰. Interestingly, we found that 3,3'-Diindolylmethane (DIM) significantly reduced the expression and activation of PDGF-D in prostate cancer cells, leading to the inhibition of cell proliferation, invasion and angiogenesis³⁵. Consistent with this finding, DIM significantly decreased the expression of PDGF-D target genes including mTOR, S6K, and

4E-BP1 phosphorylation³⁵. Recently, we also observed that DIM treatment led to the regulation of EMT marker expression and the reversal of EMT¹¹¹. For example, DIM treatment caused up-regulation of epithelial marker E-cadherin and down-regulation of mesenchymal markers ZEB1, vimentin and Slug¹¹¹. Accordingly, the morphology of cancer cells after DIM treatment changed from elongated fibroblastoid to epithelial cobblestone-like appearance, suggesting that DIM could reverse EMT to MET¹¹¹. Since PDGF-D plays a critical role in induction of EMT, the reversal of EMT by DIM is possibly mediated through the down-regulation of PDGF-D. However, further investigation is required to experimentally address whether DIM could control EMT mainly via regulation of PDGF-D.

Conclusion and overall perspectives

In conclusion, PDGF-D plays a pivotal role in the development and progression of human malignancies, which is mediated through deregulation of various cellular processes including cell proliferation, cellular apoptotic death, migration, invasion, angiogenesis and metastasis. Moreover, PDGF-D has been demonstrated to exert its oncogenic function via regulation of multiple cellular signaling pathways, leading to governing EMT that is associated with CSCs characteristics (Figure 1). Therefore, targeting PDGF-D could be a promising strategy for the treatment of cancers. Since there is no specific inhibitor for PDGF-D, it could be useful to target several signaling pathways such as PTEN, uPA that regulate PDGF-D expression as an alternative strategy. More importantly, due to non-toxic nature, inactivation of PDGF-D by natural agent DIM could be a safer approach to achieve better treatment outcome of patients diagnosed with cancers. However, further in-depth investigation is necessary to explore the mechanism(s) how PDGF-D affects tumorigenesis using transgenic mouse model. More importantly, we hope that specific PDGF-D inhibitor could be developed which will serve as an efficient chemopreventive or chemotherapeutic agent for most human malignancies that over-express PDGF-D in the future.

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Figure 1.

A proposed model for PDGF-D signaling pathway that controls the processes of EMT.

PDGF-D triggers EMT through regulation of mTOR, Notch, NF-κB, CXCR4, Bcl-2 and microRNAs. Bcl-2: B-cell lymphoma 2; CXCR4: C-X-C chemokine receptor type 4; EMT: Epithelial to mesenchymal transition; mTOR: Mammalian target of rapamycin; NF-κB: Nuclear factor kappa B. PDGF-D: Platelet-derived growth factor-D; S6K: p70 S6 ribosomal kinase; 4E-BP1: the eukaryotic translation initiation factor 4E-binding protein 1.