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Mini review

Transcriptional anti-angiogenesis therapy of human pancreatic cancer

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Abstract

Pancreatic cancer angiogenesis has been attributed to genetic and epigenetic alterations (e.g., oncogene activation and suppressor inactivation) and a chaotic tumor microenvironment (e.g., hypoxia, acidosis, free radical stress and imbalanced growth factor production). Those diverse ''upstream signal'' factors appear to converge their signaling pathways on limited sets of nuclear transcription factors (e.g., Sp1, Stat3 and NF-κB). Aberrant activities of these factors confer a tremendous survival and growth advantage to existing and/or emerging malignant cells through alteration of the expression and functions of their diverse ''downstream effector'' factors (e.g., VEGF and IL-8). Therefore, targeting a single transcription factor can affect the malignant phenotype more profoundly than just targeting any single upstream signal and/or downstream effector factor.

Keywords: Metastasis; Angiogenesis; Sp1; Therapy; Microenvironment

1. Introduction

Pancreatic ductal adenocarcinoma (referred to herein as pancreatic cancer) is one of the most lethal human cancers. Specifically, it is the fourth most common cause of cancer-related mortality in the United States and other industrialized countries [1,2]. Pancreatic cancer continues to be a major unsolved health problem, because almost all patients who have it die with conventional treatments having little impact on their disease course [2–4]. The aggressive nature of this disease is reflected by the fact that it has a propensity to metastasis when it is small and undetectable and to exhibit intrinsic resistance to cytotoxic agents and radiotherapy [2–4]. Therefore, there is an urgent need for a better understanding of the mechanisms that contribute to pancreatic cancer growth and metastasis and for the design of more effective therapies for it. This review discusses advances in the molecular pathogenesis of pancreatic cancer with a focus on the molecular biology of pancreatic cancer angiogenesis and the development of potentially effective treatments.

2. Molecular pathogenesis of pancreatic cancer

Over the past few years, our knowledge of the pathogenesis of pancreatic cancer has advanced significantly because of a rapid increase in our understanding of the molecular biology of it. Like many other malignant diseases, pancreatic cancer results from the accumulation of inherent and acquired genetic and epigenetic alterations. The multigenic nature of most pancreatic cancers is reflected by abnormalities of three broad classifications of genes: oncogenes, tumor suppressor genes and genomic maintenance genes [5,6]. Accumulated alterations of such genes are believed to occur over a predictable time course. Based on the understanding of the histological and molecular genetic profiles of pancreatic cancer, investigators have developed a progression model that describes

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pancreatic ductal carcinogenesis: the pancreatic ductal epithelium progresses from normal epithelium to increasing grades of pancreatic intraepithelial neoplasia to invasive cancer [7].

The majority of pancreatic cancers occur sporadically and have been fairly well characterized at the genetic level. Pancreatic cancer pathogenesis is apparently involved in the activation of several oncogenes and/or inactivation of various tumor suppressor genes [5,8]. Since the identification of the first notable genetic alteration of the K-*ras* oncogene, there has been an explosion in our understanding of pancreatic cancer genetics [5,8]. For examples, more than 85% of pancreatic cancers have an activating point mutation in the K-*ras* gene at a very early stage of development [9]. Also, the tumor suppressor gene *p16* is inactivated in about 95% of pancreatic cancers, and inactivation typically occurs late in pancreatic carcinogenesis. *TP53*, a well-characterized tumor suppressor gene located on chromosome 17p, is the second most frequently inactivated gene. Furthermore, *DPC4* or *SMAD4* is inactivated in 55% of pancreatic adenocarcinomas. Both *TP53* and *DPC4* inactivation are late events in pancreatic tumorigenesis. Other less common genetic alterations continue to be described in pancreatic cancer. In a comprehensive mutational analysis of 42 pancreatic ductal cancers, Rozenblum et al. [10] found that all of the tumors harbored mutations of the K-*ras* oncogene. The individual mutational frequency of the tumor suppressor genes *p16*, *TP53*, *MADH4* and *BRCA2* was 82, 76, 53 and 10%, respectively. Presumably, these alterations promote cellular proliferation, suppress apoptotic pathways, and facilitate tumor angiogenesis, invasion and metastasis.

However, the molecular mechanisms that link these genetic changes with the aggressive nature of pancreatic cancer remain poorly understood. These genetic alterations are generally perceived to eventually lead to various abnormalities in the expression and functions of a variety of growth factors and their receptors and to affect their downstream signal transduction pathways involved in the control of cell proliferation and differentiation [2,6,8,11]. For example, pancreatic cancer cells overexpress many families of growth factors and their receptors, including epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and its receptor and platelet-derived growth factor (PDGF), as well as many cytokines, such as transforming growth factor (TGF)-β, tumor necrosis factor-α, interleukin (IL)-1, IL-6 and IL-8, which enhances mitogenesis (see reviews 11–14). Pancreatic cancer also exhibits loss of responsiveness to various growth-inhibitory signals, such as members of the TGF-β family [11]. The disturbed production and function of growth-promoting and -inhibiting factors are believed to confer a tremendous survival and growth advantage to pancreatic cancer cells, as manifested by the development of angiogenic, invasive and metastatic phenotypes that are resistant to all conventional treatments.

The clinical importance of the findings described above is underscored by numerous experimental and clinical observations [11]. For example, the concomitant presence of EGF receptor (EGFR) and either EGF or TGF-β in the cancer cells is associated with disease progression and decreased survival. Overexpression of c-erbB-3, FGF-2 and TGF-β is associated with decreased survival. Moreover, dominant-negative inhibition of either EGFR or FGF receptor-1 markedly attenuates pancreatic cancer cell growth. In addition, expression of a cyclin D1 antisense construct in pancreatic cancer cells lowers cyclin D1 levels in these cells, attenuates their growth in vitro, and blocks their tumorigenicity in vivo. EGFR blockade with an anti-EGFR antibody attenuates pancreatic tumor growth, and inhibition of EGFR tyrosine kinase activity suppresses pancreatic tumor angiogenesis [15,16]. These findings are among the many that support the hypothesis that dysregulated production and function of growth factors has an important role in pancreatic cancer.

3. Angiogenic phenotype of pancreatic cancer

Tumor angiogenesis is the formation of new blood vessels from existing blood vessels and new circulating endothelial progenitor cells from bone marrow [17]. The angiogenic process is initiated by growing tumor cells, and sustained angiogenesis is dictated by the biological behavior of the tumor cells, i.e., tumor angiogenesis is tumor growth dependent. Successful induction and maintenance of angiogenesis is in turn important for sustained growth and metastasis of most solid malignancies. Both tumor cells and host cells play important roles in this process, which is often the consequence of an angiogenic imbalance, in which proangiogenic factors predominate over anti-angiogenic factors [17–21].

Numerous lines of evidence have shown that angiogenesis, as quantitated according to microvessel density (MVD), has significant clinicopathological roles in several tumors [22,23]. A very first follow-up study clearly and elegantly demonstrated that MVD in the area of the most intense neovascularization in invasive breast carcinoma is an independent, highly significant, accurate prognostic indicator for prediction of overall and relapse-free survival in patients with early-stage disease [22]. Although pancreatic cancer is not a grossly vascular tumor, it often exhibits enhanced foci of endothelial cell proliferation. Moreover, several [11,24–35] but not all [35,36] studies have reported a positive correlation between blood vessel density and disease progression in cases of pancreatic cancer, supporting the important role of angiogenesis in this disease.

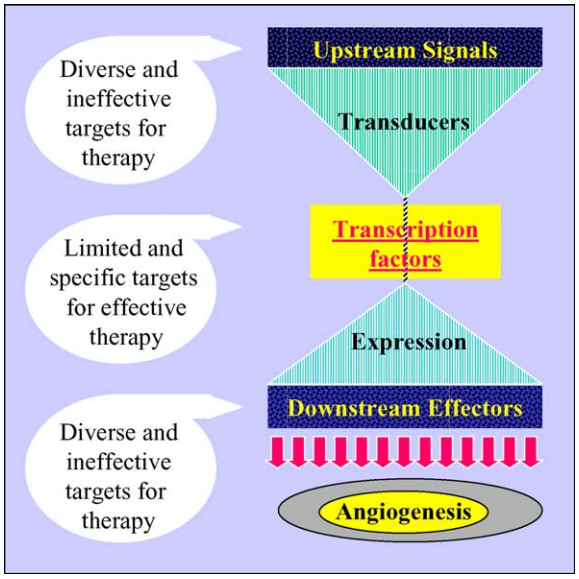
At the molecular level, numerous factors have been shown to be involved in pancreatic cancer angiogenesis. Among this growing list of growth factors, VEGF is believed to be critical for pancreatic cancer angiogenesis [11,12]. Several studies have shown that VEGF expression correlates

with MVD and disease progression [11,24,29,34,37,38]. Additionally, VEGF-C is overexpressed in pancreatic cancer and correlated with enhanced lymph node metastasis [39]. Thus, various isoforms of VEGF may contribute to the growth and metastasis of pancreatic cancer through a variety of mechanisms. Moreover, pancreatic cancers overexpress several other mitogenic growth factors that are also angiogenic, such as EGF, TGF-α, hepatocyte growth factor (HGF), FGFs (e.g., FGF-1, FGF-2 and FGF-5) and PDGF-β [11,40]. Many of these factors and others have been shown to correlate with increased vessel formation and poor prognosis in patients with pancreatic cancer; this includes expression of FGF-2 [31] and platelet-derived endothelial cell growth factor [31,34,36], mutation of K-*ras* [41] and overexpression of hypoxia-inducible factor-1α (HIF-1α) [32,42], thymidine phosphorylase [26], thrombospondin-1 [43,44] and cathepsin B and L [37]. Therefore, whereas VEGF is crucial for promoting the growth and metastasis of pancreatic cancer, other factors most likely are involved in this process, as well. Together, these factors may produce mitogenic activity in an autocrine and paracrine fashion, promoting pancreatic tumor-cell growth and angiogenesis and eventually enhancing pancreatic tumor invasion and metastasis [11–13].

Consistent with the roles of the factors described above, anti-angiogenic therapies have been demonstrated to suppress tumor growth in animal models of pancreatic cancer. Human pancreatic cancer overexpresses both IL-8 and VEGF, and specific neutralizing antibodies against IL-8 and/or VEGF have been shown to significantly reduce the growth and metastasis of human pancreatic cancer in orthotopic animal models [12,14,45,46]. The anti-angiogenic agents TNP-470 and endostatin reduce angiogenesis in tumors formed by pancreatic cancer cell lines, decreasing tumor growth and metastasis [47–49]. There are several strategies that inhibit the angiogenesis, growth and metastasis of human pancreatic cancer and improve survival in nude mouse models via targeting VEGF signaling and function, including the use of VEGF antisense oligonucleotides, VEGF-directed ribozymes, VEGF fused to diphtheria toxin, anti-VEGF antibodies, various type of interference with VEGF receptor-1 and VEGF receptor-2, a dominant-negative flk-1 and the VEGF receptor tyrosine kinase inhibitor PTK-787 [11,12]. Interestingly, celecoxib (a cyclooxygenase-2 inhibitor) and genistein (a tyrosine kinase inhibitor) suppress pancreatic cancer growth and metastasis at least in part by inhibition of VEGF expression and angiogenesis [50–52]. These findings substantiate the importance of the angiogenic process in pancreatic cancer, support the hypothesis that VEGF exerts a crucial role in this process, and raise the possibility that VEGF exerts direct effects on pancreatic cancer cells in vivo. Interference with the expression and function of other factors has also been shown to influence the angiogenesis and growth of human pancreatic cancer in animal models; these factors include dominant-negative insulin-like growth factor-I receptor (IGF-IR) [53], antithrombin III and Vitamin D-binding protein-macrophage activating factor [49,54,55], NK4, a four-kringle fragment of HGF [56] and the tyrosine kinase inhibitor of EGFR [16]. However, anti-angiogenic approaches are always profoundly more effective when combined with other strategies, such as chemotherapy and radiation therapy. For example, rapamycin and gemcitabine potentiate anti-angiogenic therapies using anti-VEGF, anti-VEGF-R2 and anti-EGFR antibodies [11]. The limited effectiveness of anti-angiogenic therapies suggests the important issue of diverse angiogenic signal and effector factors.

4. Diverse angiogenic "upstream signal" factors in pancreatic cancer

The most striking characteristic of a malignant tumor is the creation and constant evolution of its chaotic microenvironment. In essence, the creation of the microenvironment is initiated by genetic alterations and manifested by dysregulated production of growth factors and cytokines and altered status of physics and chemistry, which constitute the diverse types of intracellular and extracellular "signals" and fundamentally impact the development and progression of pancreatic cancer. For the purposes of convenience, the upstream signals are designated as consisting of genetic alterations, epigenetic changes and a microenvironment that act upon tumor cells and stromal cells and lead to changes in gene transcription. These signals are subject to spatial and temporal evolution in quality and quantity and can act as causes and/or consequences of changes in tumor biology and behavior.



The many important genetic alterations including mutations of various oncogenes and tumor suppressor genes can profoundly affect the downstream signal transduction

pathways critical to dysregulated production of growth factors and cytokines. Evidence implicates several oncogenes in increased expression of proangiogenic molecules such as VEGF, including activated forms of *Ras*, *Src*, *HER2/neu*, *Bcr/Abl*, *BCR/ABL* and *HPV-16 E6* [12]. Tumor suppressor genes have also been implicated in regulation of the expression of genes important to tumor angiogenesis; these genes include *VHL*, *TP53*, *p73*, *PTEN*, *p16*, *p63*, *BRCA1* and *Smad4/DPC4* [12]. Recent studies have consistently shown that loss or inactivation of wild-type *VHL*, *TP53* and/or *p73* is associated with increased tumor angiogenesis [57,58].

Whereas the genetic makeup of tumor cells is clearly involved in both constitutive and inducible expression of many angiogenic molecules [12,13], expression of these molecules can be drastically enhanced by numerous tumor microenvironment factors, such as hypoxia, acidosis, free radical stress and dysregulated production of various growth factors and cytokines themselves. The roles of hypoxia in angiogenesis stimulation have been well documented [12,59–62]. Interestingly, a low extracellular pH level has been shown to activate the expression of several angiogenic molecules [63,64]. For example, activation and cooperation of the nuclear factor κB (NF-κB) and activator protein-1 (AP-1) binding sites is necessary for upregulation of both IL-8 and VEGF by a low extracellular pH level [12,14]. Moreover, free radicals are integral parts of metabolism, and continuous production of them is ubiquitous in all respiring organisms. Free radical production is known to be enhanced in tumors, which may be directly related to tumor oxygen supply and metabolism [13]. As signaling molecules, many free radicals regulate the expression of genes important to tumor angiogenesis, including VEGF [12–14,65]. Finally, there are intense interactions and interregulations among different growth factors and cytokines and other factors. For example, VEGF expression can be upregulated by a number of growth factors and cytokines, including EGF, TGF-β, keratinocyte growth factor, FGF-2, FGF-4, PDGF, HGF, IL-1β, IL-1α, IL-6, tumor necrosis factor-α and IGF-1 [12,13,65].

In summary, both genetic and epigenetic changes in tumor cells and the resulting constant evolution of their microenvironment provide a huge repertoire of upstream signals, which form a complex network of cross signaling and interaction. The complexity of this network suggests not only a multitude of functional redundancy of these factors in but also an elusive nature of angiogenesis initiation and maintenance. Complete blockage of tumor angiogenesis to cure a cancer is impossible simply by interfering with one or a few angiogenic upstream signal factors. For example, mutant *Ras*-dependent VEGF expression is necessary, but not sufficient, for progressive tumor growth, suggesting a relative contribution of oncogenes to tumor angiogenesis [12,66]. This is especially true for microenvironmental factors, which are of a more stochastic nature.

5. Diverse angiogenic "downstream effector" factors in pancreatic cancer

Angiogenesis is a complex process with a stochastic nature, because it is regulated and/or influenced by diverse so-called angiogenic factors, both protein and non-protein factors. The identified angiogenic protein factors are mostly growth factors that are mitogenic to the cells in a tumor. Like other types of human cancer cells, human pancreatic cancer cells exhibit high angiogenic potential as assessed by measuring the expression and production of various growth factors and receptors that are stimuli of angiogenesis in vitro and in vivo [11]. For example, human pancreatic cancer cell lines overexpress EGFR and produce multiple ligands that bind directly to EGFR, including TGF-α, amphiregulin, heparin-binding EGF-like growth factor, betacellulin and epiregulin. These cell lines also express many other growth factors, such as FGFs and PDGF-B. Furthermore, most of the findings of in vitro studies have been confirmed by in vivo studies with human pancreatic cancer tissues that used immunohistochemistry, Northern blot analysis and in situ hybridization techniques. For example, pancreatic cancer tissue samples have been clearly shown to overexpress EGFR and six ligands that bind directly to EGFR (EGF, TGF-α, heparin-binding EGF-like growth factor, betacellulin, epiregulin and amphiregulin) as well as c-erbB-2, c-erbB-3 and c-erbB-4. These samples also overexpress FGF-2, FGF-1, keratinocyte growth factor, FGF-5, PDGF-B chain, IGF-I, the EGF-like growth factor Cripto, HGF, VEGF, all three mammalian TGF-β isoforms, bone morphogenetic protein-2 and activin βA. Overexpression of these specific receptors and their ligands in pancreatic cancer leads to the creation of aberrant paracrine and autocrine pathways that confer a distinct growth advantage to pancreatic cancer cells as well as stromal cells, particularly endothelial cells.

Many of these growth factors and cytokines have been shown to play important roles in pancreatic cancer angiogenesis. Specifically, in addition to VEGF, EGFR and HGF appear to be important to pancreatic cancer angiogenesis [11,16,67]. For example, inhibition of EGFR tyrosine kinase activity suppresses pancreatic tumor angiogenesis [67]. Other important proangiogenic factors also are overexpressed in pancreatic cancer, including certain chemokines, such as Mip3α, IL-8 and TGF-βs [11,14,45,68]. For example, expression of a soluble TβRII interfering with TGF-β action suppresses angiogenesis and the growth and metastasis of pancreatic cancer cells [11]. Additionally, recent studies have indicated that other factors, such as heme oxygenase-1 and cyclooxygenase-2 overexpression, accelerate angiogenesis in human pancreatic tumors [69,70]. Furthermore, neuropilin-1, a novel VEGF co-receptor that promotes angiogenesis, is expressed in most human pancreatic adenocarcinomas and cell lines but not in non-malignant pancreatic tissue [71]. Some important angiogenic growth factors and receptors in human pancreatic cancer are listed in Table 1.

Table 1
Angiogenic growth factors and their cognate receptor that are overexpressed in human pancreatic cancer

Factor	Receptor
VEGF-A[a]	VEGFR-1[a], VEGFR-2[a], neuropilin-1 and -2
VEGF-C[a]	VEGFR-3[a]
IFG-1[a]	IGF-1 receptor[a]
EGF[a], TGF-α, HB-EGF	EGFR[a], c-erbB2[a], c-erbB3
PDGF B chain[a]	PDGF receptor α and β[a]
FGF-1,-2, -5, -7[a]	FGFR1[a] and FGFR2[a]
HGF[a]	MET[a]
IL-6,	IL-6 receptor
IL-8	CXCR1 and CXCR2
TGF-β1, -2, -3	TGF-beta type II receptor[a]

[a] Indicates that those growth factors and/or their corresponding receptors have been demonstrated to be regulated by Sp1.

The tumor microenvironment may also serve to promote tumor angiogenesis. There are unique pancreatic cancer microenvironment factors, for example, continuous exposure to high levels of islet-cell-derived hormones, such as insulin, and growth factors, such as TGF-βs [11]. These factors may enhance pancreatic cancer angiogenesis through activation of IGF-1R [72], upregulation of matrix metalloproteinase-9 and VEGF expression [11,73,74], and suppression of pentaerythritol tetranitrate expression [75,76]. There also are notable microenvironmental factors that are seen with almost all solid tumors, including hypoxia, acidosis and free radical stress. These factors may directly and indirectly affect pancreatic cancer angiogenesis [11–13].

There are therefore multiple downstream effect factors, which form a complex network that directly regulates the angiogenic phenotype of pancreatic cancer. This network is further complicated by intensive cross-talk among the various angiogenic factors, which includes that among protein and non-protein factors. For example, the interaction between TGF-β1 and plasminogen activator inhibitor-1 influences the expression and function of each of these factors, thus promoting pancreatic cancer angiogenesis [11,77–82]. Interestingly, the angiogenic potential of TGF-βs may be enhanced by the presence of Smad4 mutations [43], which occur frequently in pancreatic cancer. Moreover, urokinase-type plasminogen activator can transactivate EGFR [83], and EGFR activation can induce the expression of VEGF and IL-8 [84,85]. Taken together, these observations suggest that multiple growth factors and cytokines as well as microenvironmental factors interact with each other at various levels and regulate pancreatic cancer angiogenesis.

6. Transcriptional control of the angiogenic phenotype in pancreatic cancer

Acquisition and maintenance of the angiogenic phenotype by pancreatic cancer are mainly determined by overexpression of proangiogenic molecules. Regulation of angiogenic molecule expression has been reported to occur at the gene transcription, translation and posttranslation levels. Transcriptional regulation of gene expression has been studied extensively, because the impact of most genetic and epigenetic factors as well as microenvironmental factors on gene expression is realized by controlling gene transcription. Although the numerous upstream factors may affect the expression and functions of a multitude of downstream "effector" angiogenic factors, only a limited number of transcription factors may actually transduce the signals to effectors. Computer-based sequence analysis of the various gene structures has revealed potential binding sites for many transcription factors in the regulatory regions of the genes key to angiogenesis. However, specificity protein 1 (Sp1), HIF-1, signal transducer and activator of transcription 3 (Stat3), NF-κB, AP-1, AP-2 and Smad4/DPC4 [12] appear to play key roles in the regulation of angiogenic gene expression, with Sp1 having a central role (Fig. 1). For example, human pancreatic cancer constitutively overexpresses Sp1 activity and VEGF [86]. Wild-type tumor suppressors such as p53, p73 and VHL have been demonstrated to physically interact with Sp1, form a complex, and block Sp1 binding to the promoter of VEGF, thus inhibiting Sp1-mediated VEGF expression [12].

Sp1 is a sequence-specific DNA-binding protein that is important to the transcription of many cellular and viral genes that contain GC boxes in their promoter [87–90]. Additional transcription factors similar to Sp1 in their structural and transcriptional properties (Sp2, Sp3, Sp4, Sp5, Sp6, Sp7, Sp8 and Sp9) have been cloned, thus forming the Sp1 multigene family [87–90]. Recent studies have also shown that Sp1 may regulate the expression of genes that are intimately involved in many other aspects of cancer biology, such as angiogenesis, invasion and resistance to apoptosis. Accumulating evidence has suggested that the members of the Sp1 family are involved in multiple aspects of angiogenesis and that this involvement appears to extend to all cell types involved in vessel formation [12].

Sp1-binding sites have been found in the promoters of multiple genes encoding angiogenic molecules, including VEGF, FGFs, EGFR and IGF-IR, etc. (see Table 1). For example, many genetic alterations affect VEGF expression through modulation of the transcriptional activity of Sp1. These include alterations of tumor suppressor genes, such as *VHL*, *TP53* and *p73*, and oncogenes, such as *Ras*, *Src* and *HER2/neu* [12]. In addition, VEGF expression can be regulated through modulation of Sp1 activity by tumor microenvironment factors, such as free radicals, hypoxia, growth factors and cytokines [12]. Moreover, these factors as well as genetic and epigenetic changes also activate other major transcription factors, such as HIF-1, Stat3 and NF-κB [11–13,68,91,92].

Interestingly, optimal transcriptional gene activation appears to require the activation and cooperation of different transcription factors. In most cases, physical interaction and

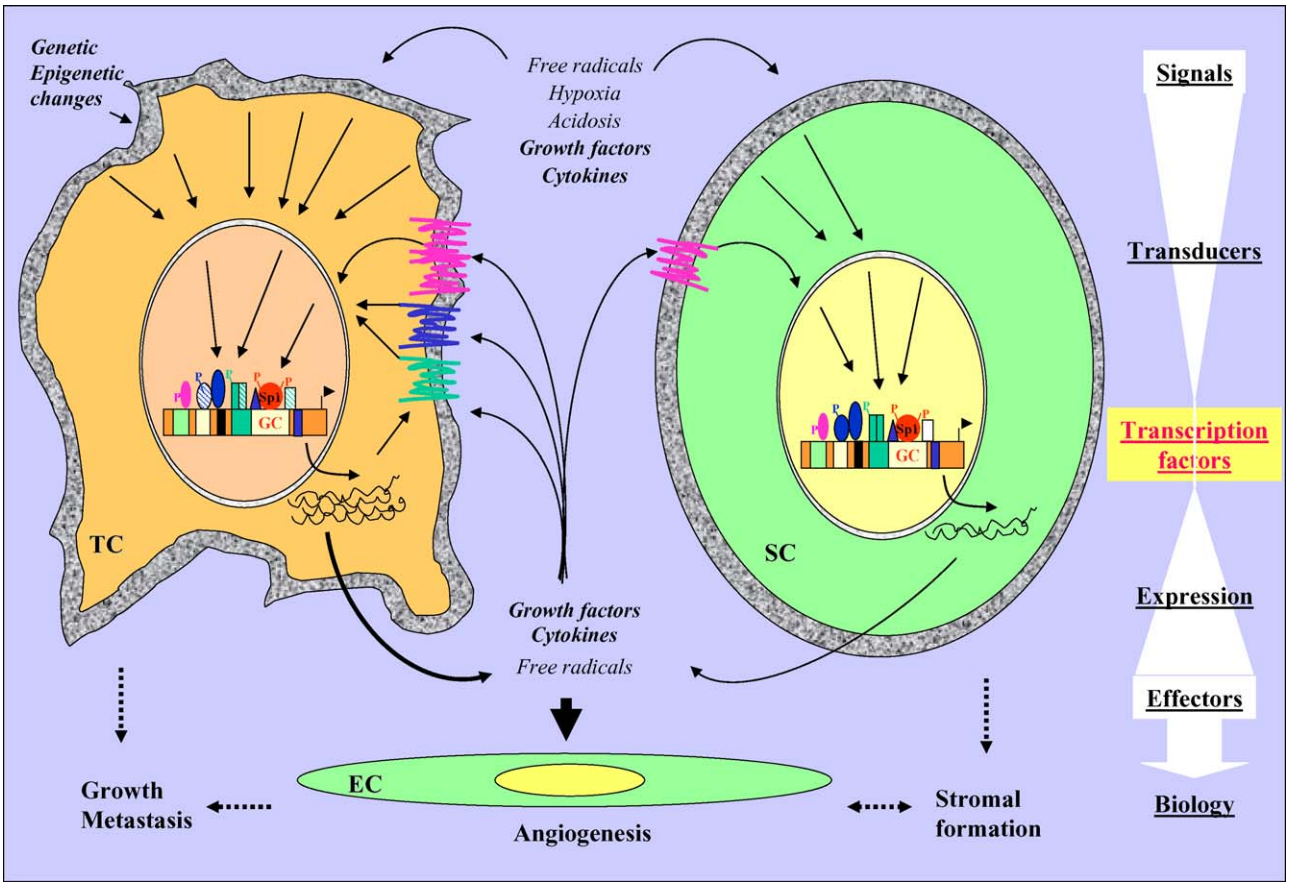


Fig. 1. Chaotic nature and potential targets of tumor angiogenesis. Tumor angiogenesis is initiated by genetic and epigenetic changes in tumor cells (TC) and subsequent chemical and physical alterations of their microenvironment. Those protein and non-protein factors constitute the diverse angiogenesis upstream signals, which are under spatial and temporal change. Conversely, there also are diverse effector factors directly affect the process of angiogenesis, such as endothelial cell (EC) proliferation and differentiation, and tumor stromal formation. Those down stream effectors, most of which are in effect signal factors, are also under constant change in quality and quantity. Both signal and effector factors are subject to further alteration due to continuing genotypic and phenotypic changes of tumor cells as well as stromal cells (SC). Any strategies targeting those individual factors deem unsuccessful. However, it appears that there are only a few transcriptional factors that critically transduce signals from upstream signal factors to the generation of downstream effector factors. Those transcriptional factors may serve as effective targets for anti-angiogenic cancer therapy.

functional cooperation between Sp1 and other transcription factors is crucial, suggesting a central role for Sp1 in the expression of multiple angiogenic factors. Like genetic alterations, a wide variety of tumor microenvironment factors can activate the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/Akt pathways. These factors include free radicals, hypoxia, acidosis and a number of growth factors, such as IGF-1, HGF, FGF, EGF, VEGF and PDGF [12]. Activation of the MAPK and/or PI3K/Akt pathways leads to induction of Sp1 transcriptional activity as well as AP-1, NF-κB and Stat3 transcriptional activity [12]. For example, full induction of VEGF expression stimulated by UVB radiation requires the cooperation of Sp1 with AP-1 [93]. Major transcription factors and their main targets in the regulation of pancreatic cancer angiogenesis are listed in Table 2.

Finally, many transcription factors exhibit constitutive and inducible activation and may play different roles at different stages of pancreatic cancer development and progression. In the early stages of pancreatic cancer growth, genetic alterations may predominantly contribute to constitutive activation of transcription factors. However, in the late stages of pancreatic cancer progression, important stress factors, such as hypoxia and acidosis, which are frequently encountered in the tumor microenvironment, may further upregulate the activities of various transcription factors. Therefore, at advanced stages, uncontrolled tumor growth

Table 2
Major transcription factors and downstream targets that are involved in the regulation of angiogenic phenotype of pancreatic cancer

Factor	Downstream target
Sp1	VEGF-A, VEGFR-1, VEGFR-2, HGF, FGFR, EGFR, c-erbB2, c-erbB3, IGF-IR, IGF-1, MET, NOS III
STAT3	VEGF-A, VEGFR-1, VEGFR2, NOS II
NF-κB	VEGF-A, IL-8, IL-6, NOS II
HIF-1	VEGF-A, NOS II
AP-1	VEGF, IL-8, NOS II
TP53[a]	VEGF-A, NOS II
vHL[a]	VEGF-A, HIF

[a]TP53 and vHL are considered as negative regulators.

and the consequent development of a stressful microenvironment may accelerate tumor angiogenesis, growth and metastasis [2,12,13]. Understanding the expression and regulation of these transcription factors might shed more light on the molecular pathophysiology of pancreatic cancer and suggest new targets for preventive and treatment approaches for pancreatic cancer.

7. Transcriptional anti-angiogenic therapy: logic, promise and challenges

There have been numerous attempts at treating pancreatic cancer as well as other types of cancer by targeting one or more factors that could function as signal and/or effector factors in tumor angiogenesis. That the outcome is usually highly disappointing, as attested to by preclinical experimentation and clinical trials, is not surprising [11,94–97]. The apparent failure of these attempts suggests two important insights. First, angiogenesis itself is driven by tumor growth. Effective anti-angiogenic therapy may have to target both compartments of a tumor: the tumor cells (the driving force for angiogenesis initiation and maintenance) and the stromal cells (mainly endothelial cells). Targeting the stroma may only temporarily limit tumor growth before the next wave of rapid growth occurs, when tumor cells establish their alternative or alternatives. Although it is practically impossible at the present time, effective control of tumor-cell growth would completely shut down angiogenesis. This was unequivocally demonstrated in a transgenic model of melanoma and breast cancer, showing that withdrawal of a tumorigenic oncogene induces tumor regression and complete collapse of its existing vasculature [98–100]. Second, the angiogenic process consists of various stochastic elements, from multiple signal and effector factors and their spatial and temporal changes in quantity and quality to constant evolution of the tumor-cell genotype and phenotype and their diverse interactions with the changing tumor microenvironment. There is convincing evidence suggesting that some factors, such as VEGF, are crucial to regulation of tumor angiogenesis and could be very important targets for antitumor therapy [11,12]. However, there is no proof that any single factor, including VEGF, is essential for tumor angiogenesis and a completely effective therapeutic target for a cure. Therefore, for effective therapy, just targeting any one or a few of these factors would be arbitrary, whereas identifying and then targeting elements that are of a less stochastic nature would be more logical.

Although the vast number of upstream signal factors may affect the expression and functions of a multitude of downstream ''effector'' angiogenic factors, only a limited number of transcription factors can actually transduce the signals to effectors. These transcription factors may be elements that function predictably during tumor angiogenesis. Therefore, targeting a single transcription factor may have a more profound effect on the malignant phenotype of pancreatic cancer than just targeting any single signal and/or effector factor.

In summary, diverse upstream signals, including genetic and epigenetic factors as well as tumor microenvironment factors, use one or more pathways to modulate the expression and function of several transcription factors that are critical to the transcriptional regulation of various genes key to tumor angiogenesis. The transcriptional activities of these transcription factors are mainly subject to the Ras/raf/extracellular signal-regulated kinase/p42/p44 MAPK and PI3K/Akt signaling pathways and control the expression of their numerous downstream target genes. Searching for the transcription factors critical to angiogenesis and testing them as targets for anti-angiogenic therapy rather than continued targeting of single signal and/or effector factors would be more fruitful. Although critical issues remain regarding how to identify critical transcription factors and target them effectively for the treatment of pancreatic cancer, our opinion is that the proteins in the Sp1 family, among several others, are of great promise and may ultimately be ideal therapeutic targets for the treatment of pancreatic cancer.

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