Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease. Although the specific mechanisms that dictate its biological aggressiveness are not clearly established, it is characterized by a variety of molecular alterations as well as by the overexpression of mitogenic and angiogenic growth factors and their receptors. PDACs also express high levels of vascular endothelial growth factor (VEGF). Recent studies indicate that suppression of VEGF expression attenuates pancreatic cancer cell tumorigenicity in a nude mouse model, and that VEGF can exert direct mitogenic effects on some pancreatic cancer cells. These findings suggest that cancer cell derived VEGF promotes pancreatic cancer growth in vivo via a paracrine angiogenic pathway and an autocrine mitogenic pathway, and provide novel opportunities for therapeutic intervention in this deadly disease.

Carcinoma of the pancreas: An overview

Pancreatic ductal adenocarcinoma (PDAC) is responsible for over 20% of deaths due to gastrointestinal malignancies, making it the fourth most common cause of cancer related mortality in the United States and other industrialized countries. The prognosis of patients with PDAC is extremely poor, with overall 5-year survival rates that are less than 1%, one-year overall survival of 12%, and a median survival of 6 months [2]. Survival is often limited to patients who had surgical resection at an early stage of the disease. However, the diagnosis of PDAC is often established at an advanced stage, precluding patients from undergoing tumor resection in spite of limited results with other treatment modalities [3]. These dismal statistics are due to the tumor’s propensity to metastasize when small and undetectable, the advanced stage at which many patients first develop symptoms, and the intrinsic resistance of pancreatic cancer cells to cytotoxic agents and radiotherapy [3–5]. PDAC may be an even more serious problem in the future since its incidence increases after age 50 and the general population world-wide is aging.

There is, therefore, an urgent need for an improved understanding of the mechanisms that contribute to pancreatic tumor growth and metastasis, and for the design of therapies for this disorder that are more effective than current regimens. This review will cover in a brief manner the molecular biology of pancreatic cancer, and will then focus on various aspects of vascular endothelial growth factors in angiogenesis in general and in relation to PDAC in particular.

Molecular biology of pancreatic cancer

A plethora of genetic mutations have been described in the cancer cells of PDAC patients. The most frequent alterations (approximate frequency indicated in parenthesis) include mutations in the K-ras oncogene (90%), the p53 (85%) and Smad4 (30%) tumor suppressor genes, and the p16 (85% mutated and 15% silenced epigenetically) cell cycle inhibitory gene [6,7]. Together, these alterations promote cellular proliferation, suppress apoptotic pathways, and facilitate tumor spread and metastasis. In addition, there is overexpression of multiple tyrosine kinase...
receptors and their ligands which enhances mitogenesis, and loss of responsiveness to the growth-inhibitory signals of members of the transforming growth factor beta (TGF-β) family [6,7], which contribute in a significant manner to the biological aggressiveness of PDAC.

It is well established that human pancreatic cancer cell lines overexpress the epidermal growth factor (EGF) receptor (EGFR) and produce multiple ligands that bind directly to EGFR, including transforming growth factor-alpha (TGF-α), amphiregulin, heparin-binding EGF-like growth factor (HB-EGF), betacellulin and epiregulin [8–12]. These cell lines also express other growth factors such as fibroblast growth factors (FGFs) and platelet-derived growth factor (PDGF) B chain [13–16]. However, expression of receptors and ligands in cell lines does not necessarily indicate parallel alterations in PDAC in vivo. Therefore, studies using human tissues have been of vital importance in this regard. Studies using immunohistochemistry, Northern blot analysis and in situ hybridization techniques, have demonstrated that PDAC tissue samples overexpress EGFR and six ligands that bind directly to EGFR (EGF, TGF-α, HB-EGF, betacellulin, epiregulin and amphiregulin), as well as c-erb-B2, c-erb-B3, and c-erb-B4 [10,11,17–19]. These cancers also overexpress basic fibroblast growth factor (FGF-2), acidic FGF (FGF-1), keratinocyte growth factor (KGF), FGF-5, PDGF B chain (but not A chain), insulin-like growth factor-I (IGF-I), the EGF-like growth factor Cripto, hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), all 3 mammalian transforming growth factor beta (TGF-β) isoforms, bone morphogenetic protein-2 (BMP-2) and activin βA [14,15,20–29]. Many, but not all of the corresponding receptors are concomitantly overexpressed. For example, there is overexpression of PDGF receptor α and β, the IGF-1 receptor, MET (the receptor that binds HGF), the 2 Ig-like form of type I FGF receptor (FGFR-1), and the type II TGF-β receptor (TβRII) but not the insulin receptor [16,21,23,26,30–33]. IGF-II and insulin are not overexpressed in PDAC [21], whereas the type I TGF-β receptor (TβRI) is under-expressed [31–33]. Thus, there is selective overexpression of specific receptors and their ligands in PDAC, and this concomitant overexpression leads to the creation of aberrant paracrine and autocrine pathways that confer a distinct growth advantage to pancreatic cancer cells.

The clinical importance of the above observations is underscored by numerous observations. For example, the concomitant presence in the cancer cells of EGFR and either EGF or TGF-α is associated with disease progression and decreased survival of PDAC patients [34]. Overexpression of c-erbB3 [19], FGF-2 [20] or TGF-β [35] is associated with decreased patient survival. The aberrant cytoplasmic localization of amphiregulin [36] is also associated with decreased patient survival. Dominant negative inhibition of either EGF or FGFR-1 markedly attenuates pancreatic cancer cell growth [37–39]. 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 [40]. EGFR blockade with an anti-EGFR antibody attenuates pancreatic tumor growth, and inhibition of EGFR tyrosine kinase activity suppresses pancreatic tumor angiogenesis [41,42]. Together, these findings are among many that support the hypothesis that tyrosine kinase receptors and ligands have an important role in PDAC.

**VEGF family of growth factors and their receptors**

VEGF-A, also called "vascular permeability factor", is a homodimeric heparin-binding glycoprotein [43–45]. Five major VEGF-A isoforms having 121, 145, 165, 189 and 206 amino acid residues, respectively, arise as a result of alternative splicing from a single gene [46,47]. VEGF-A121 and VEGF-A145 are usually secreted while VEGF-A189 and VEGF-A206 are almost completely sequestered in the extracellular matrix [47]. VEGF-A165 is half secreted and half bound to the cell surface and the extracellular matrix [48]. All 5 isoforms are mitogenic toward vascular endothelial cells and induce vascular permeabilization. Additional VEGF isoforms and VEGF-related genes have been identified, including VEGF-B [49,50], VEGF-C [51], VEGF-D [52], VEGF-E [53] and placenta growth factor [54]. Direct evidence for the role played by VEGF-A in embryonic vasculogenesis and angiogenesis was also demonstrated in VEGF-A gene knockout studies [55,56], in which loss of a single VEGF-A allele in mice resulted in embryonic lethality between day 11 and 12. Angiogenesis and blood-Island formation were impaired, resulting in severe developmental anomalies. This heterozygous lethal phenotype is indicative of the tight dose-dependent regulation of embryonic vessel development by VEGF-A [55,56]. VEGF-A is also required for the cyclical blood vessel proliferation in the female reproductive tract and for longitudinal bone growth and endochondral bone formation in postnatal development [43]. Together, these observations indicate that VEGF-A has an important role in embryogenesis, development, and tissue remodeling.

VEGF-A stimulates endothelial cell proliferation through binding to two related tyrosine kinase receptors, VEGFR-1 (flt-1) VEGFR-2 (flk-1/KDR), on the surface of endothelial cells, with most of the mitogenic effects taken to occur via VEGFR-2 [57–59] (57–59). A third high affinity VEGF receptor, termed VEGFR-3 (Flt4), is expressed in lymphatic vessels [60,61]. It is activated by VEGF-C, which can be processed to a form that also binds to VEGFR-2 [57–61]. Furthermore, placenta growth factor and VEGF-B bind only VEGFR-1, whereas VEGF-D, like VEGF-C, interacts
with both VEGFR-2 and VEGFR-3 [57–61]. However, VEGF-E binds only to VEGFR-2 [59]. All three VEGFRs are class III transmembrane protein tyrosine kinases that possess seven immunoglobulin-like sequences in their extracellular domains and a kinase insert in their intracellular domains [57–61]. In addition, neuropilin-1 (Np-1), a neuronal guidance molecule for axons in the developing nervous system, also acts as a co-receptor for VEGF-A165 (but not for VEGF-A121), PlGF-2, VEGF-B and VEGF-E [62]. Np-1 is a non-tyrosine kinase transmembrane protein whose overexpression in transgenic mice is associated with various abnormalities, including excess capillary and blood vessel formation [63]. The closely related neuropilin-2 (Np-2) also binds VEGF-A165 (but not VEGF-A121), as well as VEGF-A145 and PlGF-2, strongly implying that both Np-1 and Np-2 in angiogenesis [62–64].

Gene knockout studies have shown that both VEGFR-1−/− and VEGFR-2−/− mice die in utero between day 8.5 and 9.5 [65,66]. In VEGFR-1−/− mice, endothelial cells developed in both embryonic and extra-embryonic sites but failed to organize into normal vascular channels [65]. In VEGFR-2−/− mice, hematopoietic precursors were severely reduced, yolk-sac blood islands were absent, organized blood vessels failed to develop throughout the embryo or the yolk sac [66]. Furthermore, double knockouts for Np-1 and Np-2 die in utero between day 8.5 and 9.5 [67]. They exhibit avascular yolk sacs, and mice that are deficient for Np-1 but heterozygous for Np-2, or deficient for Np-2 but heterozygous for Np-1, die at day 10 to 10.5 and exhibit diffuse vascular abnormalities that are more marked than either Np-1 or Np-2 single knockouts [67]. Together, these observations suggest that VEGFR-1 and VEGFR-2 are essential for embryonic vasculature development, whereas VEGFR-3 is essential for lymphangiogenesis, and that Np-1 and Np-2 are as important as the other components of the VEGF pathway in embryonic angiogenesis.

**Angiogenesis in cancer**

Tumor angiogenesis is often the consequence of an angiogenic imbalance in which pro-angiogenic factors predominate over anti-angiogenic factors [68–71]. Furthermore, angiogenesis is essential for growth and metastasis of most solid malignancies, and VEGF-A is believed to be critical for tumor angiogenesis [72,73]. Thus, secretion of bioactive VEGF-A by cancer cells may be directly involved in tumor progression [43]. For example, ovarian cancer cells secrete large amounts of bioactive VEGF-A that may play a crucial role in the genesis of ascitic fluid accumulation, angiogenesis and tumor induced immunosuppression in ovarian cancer patients [74]. In high grade gliomas, bioactive VEGF-A secreted by the glioma cells may account for the histopathological and clinical features of these tumors, including such characteristics as marked tumor angiogenesis and increased cerebral edema [75,76].

VEGF-A is expression is induced by multiple mechanisms. These include mutant K-ras and mutant p53, the von Hippel Lindau gene product, growth factors such as FGF-2 and TGF-β, hypoxia, and transcription factors such as hypoxia inducible factor 1 alpha and SP1 [77–81]. VEGF-A is up-regulated in many tumors including mammary, colorectal, renal, liver, ovarian and gastric carcinomas and gliomas [43], and its overexpression has been correlated with poor prognosis. For example, breast cancer patients with metastatic disease whose tumors exhibit increased angiogenesis have a worse prognosis than the corresponding patients whose tumors do not exhibit increased angiogenesis [82]. Furthermore, suppression of VEGF-A functions inhibits tumor growth in animal models as demonstrated with a dominant negative VEGFR-2, soluble VEGFR-1, neutralizing anti-VEGF-A antibody, VEGF-A anti-sense expression, anti-VEGF-1 or anti-VEGF-2 ribozymes, tyrosine kinase inhibitors of VEGFR-2, and anti-VEGF-2 antibodies [83–92].

**Role of VEGF in pancreatic cancer angiogenesis**

Although PDAC is not a grossly vascular tumor, this malignancy often exhibits enhanced foci of endothelial cell proliferation. Moreover, several [24,93,94], but not all [95] studies, have reported a positive correlation between blood vessel density, tumor VEGF-A levels, and disease progression in PDAC, raising the possibility that VEGF-A may have an important role in this disease. However, PDACs overexpress multiple additional mitogenic growth factors which are also angiogenic (Table 1), such as EGF, TGF-α, HGF, FGFs such as FGF-1, FGF-2, and FGF-5, and PDGF-beta [6,96]. Therefore, while VEGF-A is of crucial importance in promoting the growth and metastasis of pancreatic cancer cells in PDAC, other factors are most likely also involved in this process. Nonetheless, it has been demonstrated that pancreatic cancer cells secrete biologically active VEGF-A [25], and the cancer cells in PDAC as well as pancreatic cancer cell lines sometimes express VEGFR-1 and/or VEGFR-2 [97]. Moreover, some of these cells may be growth stimulated by VEGF-A in cell culture [97,98], and the major angiogenic agent toward human dermal microvascular endothelial cells (HDMEC) that is produced by T3M4 and PANC-1 human pancreatic cancer cells is VEGF-A, since the mitogenic activity of conditioned medium from these cells can be nearly completely suppressed by neutralizing anti-VEGF-A antibodies [99]. Together, these observations suggest that by promoting angiogenesis VEGF-A enhances tumor spread and metastasis in this malignancy.

In support of the above conclusion, it has been demonstrated that anti-angiogenic therapy is effective at sup-
pressing tumor growth in animal models of PDAC. Thus, the anti-angiogenic agent TNP-470 reduces neoangiogenesis in tumors formed by pancreatic cancer cell lines, and decreases tumor growth and metastasis [99]. Suppression of VEGF-A expression with a VEGF-A antisense construct and with a VEGF directed ribozyme markedly attenuates tumorigenicity in nude mice and formation of hepatic metastases [25,100]. VEGF-A fused to diphtheria toxin (DT-VEGF) internalizes in target cells via VEGFRs, inhibits protein synthesis, and suppresses the growth of HUVEC endothelial cells, thereby decreasing the volume and microvessel density in tumors formed by pancreatic cancer cells [101]. Adenoviral vectors carrying sequences encoding soluble VEGFR-1 and VEGFR-2 [102,103], or the VEGFR tyrosine kinase inhibitor PTK 787 [104], also inhibit the growth of growth and/or metastasis of pancreatic cancers in mouse models. These findings underscore the importance of the angiogenic process in PDAC, support the hypothesis that VEGF-A exerts a crucial role in this regard, and raise the possibility that VEGF-A may exert direct effects on pancreatic cancer cells in vivo.

VEGF-A can also act as a survival factor for endothelial cells, rendering these cells more radioresistant [105]. It can also promote the survival of leukemic cells, certain tumor cells and hematopoietic stem cells [106–108]. In addition, VEGF-C is also overexpressed in PDAC, and this overexpression has been correlated with enhanced lymph node metastasis [109]. Thus, various members of the VEGF family of ligands may contribute to the growth and metastasis of pancreatic cancer cells through a variety of mechanisms.

**Additional mechanisms for promoting pancreatic cancer angiogenesis**

Although VEGF appears to be of paramount importance for the angiogenic process in PDAC, these cancers express many other pro-angiogenic factors (Table 1). As in the case of VEGF, some of these growth factors activate tyrosine kinase receptors that are expressed in endothelial cells within the pancreatic tumor mass, such as EGFR [17]. The importance of tyrosine kinase receptors other than VEGFR in pancreatic cancer angiogenesis is underscored by recent observations that inhibition of EGF tyrosine kinase activity suppresses pancreatic tumor angiogenesis [42], and that NK4, an antagonist that is composed of the N-terminal hairpin and subsequent four-kringle domains of HGF, is a competitive antagonist for HGF that potently inhibits angiogenesis in tumors formed by SUIT-2 pancreatic cancer cells [110].

Other pro-angiogenic factors that are overexpressed in PDAC include certain chemokines such as Mip3α and interleukin-8 (IL-8), which activate G-protein coupled receptors [111–113]. By contrast, TGF-βs activate serine-threonine kinase receptors [114]. The importance of TGF-βs are pro-angiogenic factors in PDAC is underscored by the recent observation that expression of a soluble TβRII in pancreatic cancer cells interferes with TGF-β actions, attenuates tumor growth and metastasis, and suppresses tumor angiogenesis [Rowland-Goldsmith, 2001 #905; Rowland-Goldsmith MA, 2002 #2548].

Often, there is evidence for cross-talk between the various angiogenic factors. For example, TGF-β1 and plasminogen activator inhibitor-1 (PAI-1) are overexpressed in PDAC [117,118], TGF-β1 induces PAI-1 expression in pancreatic cancer cells [119], and both TGF-β1 and PAI-1 and can promote angiogenesis in vivo [120–122]. TGF-βs are initially released as latent molecules that form complexes with latent binding protein (LTBP), and their biological effectiveness is dependent on their activation by such proteins as plasmin, uPA and its receptor, the insulin-like growth factor II (IGF-2) receptor, and tissue transglutami-

---

**Table 1: Examples of Angiogenic Growth Factors that Are Overexpressed in Human Pancreatic Cancer and their Cognate Receptors**

| Growth Factors Activating Tyrosine Kinase Receptors | Receptor |
|---------------------------------------------------|----------|
| VEGF-A, VEGF-C, EGF, TGF-α, HB-EGF, FGF-1, -2, -5 | VEGFR-1 and VEGFR-2, VEGFR-3 |
| PDGF B chain, IGF-1, Hepatocyte growth factor | EGF receptor, FGF receptors, types 1 and 2, PDGF receptors α and β, IGF-1 receptor |
| Growth Factors that Activate Serine-Threonine Kinase Receptors | MET |
| TGF-β1, -2, -3 | Type II TGF-β receptor |
| Pro-Angiogenic Chemokines | CXCR1 and CXCR2 |
| IL-8 | CCR6 |
| Mip 3α | |

---

Molecular Cancer 2003, 2 http://www.molecular-cancer.com/content/2/1/8
nase [123,124]. The IGF-2 receptor, as well as uPA and its receptor are overexpressed in PDAC [125,126], and pancreatic cancer cell lines express tissue transglutaminase [127]. Furthermore, uPA and its receptor, as well as tissue transglutaminase, have been implicated in the angiogenic process [128,129], and the angiogenic potential of TGF-βs may be enhanced by the presence of Smad4 mutations [130], which are frequent in PDAC. uPA can transactivate EGFR [131], and EGFR activation can induce the expression of VEGF and the pro-angiogenic chemokine interleukin-8 [132,133]. Taken together, these observations suggest that multiple pathways interact to enhance angiogenesis in PDAC.

The pancreatic microenvironment may also serve to promote tumor angiogenesis [134]. In addition, as a consequence of the existence of a continuous intra-pancreatic portal circulation, pancreatic cancer cells may be exposed to high levels of islet cell derived hormones such as insulin and growth factors such as TGF-βs [135]. High insulin levels bind and activate the IGF-1 receptor, which can then promote angiogenesis [136,137]. Furthermore, islet cell derived TGF-βs may enhance matrix metalloprotease-9 (MMP-9) and VEGF expression in PDAC [31,138], and suppress PTEN expression [139]. MMP-9 enhances tumor angiogenesis [140] whereas PTEN, a phosphatase with a sequence of the existence of a continuous intra-pancreatic micro-angiogenesis and overexpress multiple pro-angiogenic factors. VEGF and related ligands represent a crucial component of this pro-angiogenic switch, as evidenced by the presence of high levels of VEGF in ascitic fluid of PDAC patients [142], the correlation between high serum VEGF levels and disease recurrence post-operatively [143], and the observation that high VEGF-2 levels are associated with a worse prognosis in this disease [144]. Therefore, mechanisms that target VEGF and the various pathways that enhance the angiogenic process in PDAC [145] may ultimately be of great therapeutic benefit in patients with unresectable disease as well as following surgery to prevent disease recurrence.

Conclusion

PDAC is a biologically aggressive malignancy that has a propensity to spread locally and metastasize distally. While not grossly vascular, these cancers exhibit foci of micro-angiogenesis and overexpress multiple pro-angiogenic factors. VEGF and related ligands represent a crucial component of this pro-angiogenic switch, as evidenced by the presence of high levels of VEGF in ascitic fluid of PDAC patients [142], the correlation between high serum VEGF levels and disease recurrence post-operatively [143], and the observation that high VEGF-2 levels are associated with a worse prognosis in this disease [144]. Therefore, mechanisms that target VEGF and the various pathways that enhance the angiogenic process in PDAC [145] may ultimately be of great therapeutic benefit in patients with unresectable disease as well as following surgery to prevent disease recurrence.

References

1. Warshaw AL and Fernandez-del Castillo C. Pancreatic carcinoma. N Engl J Med 1992, 326(7):455-65
2. Parker SL. Cancer statistics, 1997. CA Cancer J Clin 1997, 47(1):5-27
3. Bramhall SR and Neoptolemos JP. Adjutant chemotherapy in pancreatic cancer. Int J Pancreatol 1997, 21(1):59-63
4. Abrams RA. Role of radiation therapy in the management of the patient with pancreatic cancer. Oncology (Huntingt) 1996, 10(9 Suppl):13-7
5. Kuvshinoff BW and Bryer MP. Treatment of resectable and locally advanced pancreatic cancer. Cancer Control 2000, 7(5):428-36
6. Korc M. Role of growth factors in pancreatic cancer. Surg Oncol Clin N Am 1998, 7(1):35-41
7. Kern SE. Molecular genetic alterations in ductal pancreatic adenocarcinomas. Med Clin North Am 2000, 84(3):691-5
8. Korc M, Meltzer P and Trent J. Enhanced expression of epidermal growth factor receptor correlates with alterations of chromosome 7 in human pancreatic cancer. Proc Natl Acad Sci U S A 1986, 83(14):5141-4
9. Smith JJ, Derynck R and Korc M. Production of transforming growth factor alpha in human pancreatic cancer cells: evidence for a superagonist autocrine cycle. Proc Natl Acad Sci U S A 1987, 84(21):7567-70
10. Ebelt M. Induction and expression of amphiregulin in human pancreatic cancer. Cancer Res 1994, 54(15):3959-62
11. Kobrin MS. Induction and expression of heparin-binding EGF-like growth factor in human pancreatic cancer. Biochem Biophys Res Commun 1994, 202(3):1705-9
12. Yokoyama YH, Kobrin MS, Ebert M, Friess H, Buchler MW and Korc M. Betacellulin, a member of the EGF family is overexpressed in human pancreatic cancer. Int J Oncol 1995, 5:825-829
13. Kornmann MH, Beger G, Fritz R and Korc M. Role of fibroblast growth factors and their receptors in pancreatic cancer and chronic pancreatitis. Pancreas 1998, 17(2):169-75
14. Kornmann M. Fibroblast growth factor-5 stimulates mitogenic signaling and is overexpressed in human pancreatic cancer: evidence for autocrine and paracrine actions. Oncogene 1997, 15(12):1417-24
15. Siddiqi I. Increased expression of keratinocyte growth factor in human pancreatic cancer. Biochem Biophys Res Commun 1995, 215(1):309-15
16. Ebelt M. Induction of platelet-derived growth factor A and B chains and over-expression of their receptors in human pancreatic cancer. Int J Cancer 1995, 62(5):529-35
17. Korc M. Overexpression of the epidermal growth factor receptor in human pancreatic cancer is associated with concomitant increases in the levels of epidermal growth factor and transforming growth factor alpha. J Clin Invest 1992, 90(4):1352-60
18. Yamanaka Y. Overexpression of HER2/neu oncogene in human pancreatic carcinoma. Hum Pathol 1993, 24(10):1127-34
19. Friess H. Enhanced erbB-3 expression in human pancreatic cancer correlates with tumor progression. Clin Cancer Res 1995, 1(11):1413-20
20. Yamanaka Y. Overexpression of acidic and basic fibroblast growth factors in human pancreatic cancer correlates with advanced tumor stage. Cancer Res 1993, 53(21):5289-96
21. Bergmann U. Insulin-like growth factor I overexpression in human pancreatic cancer: evidence for autocrine and paracrine roles. Cancer Res 1995, 55(10):2007-11
22. Friess H and Cripto, a member of the epidermal growth factor receptor family, is over-expressed in human pancreatic cancer and chronic pancreatitis. Int J Cancer 1994, 56(5):668-74
23. Ebelt M. Coexpression of the c-met proto-oncogene and hepatocyte growth factor in human pancreatic cancer. Cancer Res 1994, 54(22):5775-8
24. Itakura J. Enhanced expression of vascular endothelial growth factor receptor in human pancreatic cancer correlates with local disease progression. Clin Cancer Res 1997, 3(8):1309-16
25. Luo J. Pancreatic cancer cell-derived vascular endothelial growth factor is biologically active in vivo and enhances tumorigenicity in vivo. Int J Cancer 2001, 92(3):361-9
26. Friess H. Enhanced expression of the type II transforming growth factor beta receptor in human pancreatic cancer cells without alteration of type III receptor expression. Cancer Res 1993, 53(12):2704-7
27. Kleeff J. Bone morphogenetic protein 2 exerts diverse effects on cell growth in vitro and is expressed in human pancreatic cancer in vivo. Gastroenterology 1999, 116(5):102-16
28. Kleeff J. Concomitant over-expression of activin/inhibin beta subunits and their receptors in human pancreatic cancer. Int J Cancer 1998, 77(6):680-8
29. Bergmann U. Increased expression of insulin receptor substrate-1 in human pancreatic cancer. Biochem Biophys Res Commun 1996, 220(3):886-90
Molecular Cancer 2003, 2
http://www.molecular-cancer.org/content/2/1/8

30. Kobrin MS Aberrant expression of type I fibroblast growth factor receptor in human pancreatic adenocarcinomas. Cancer Res 1993, 53(20):7811-6.
31. Wagner M Enhanced expression of the type II transforming growth factor-beta receptor is associated with decreased survival in human pancreatic cancer. Pancreas 1999, 19(4):370-6.
32. van Z Presence of two signaling TGF-beta receptors in human pancreatic cancer correlates with advanced tumor stage. Dig Dis Sci 1997, 42(10):2054-63.
33. Wagner M Transfection of the type I TGF-beta receptor restores TGF-beta responsiveness in pancreatic cancer. Int J Oncol 1997, 10(5):255-60.
34. Yamanaka Y Coexpression of epidermal growth factor receptor and ligands in human pancreatic cancer is associated with enhanced tumor aggressiveness. Anticancer Res 1993, 13(3):565-9.
35. Friess H Enhanced expression of transforming growth factor beta isoforms in pancreatic cancer correlates with decreased survival. Gastroenterology 1993, 105(6):1846-56.
36. Yokoyama MEM, Funatomi H, Fries H, Büchler MW, Johnson GR and Korc M Amphiregulin is a potent mitogen in human pancreatic cancer cells: correlation with patient survival. Int J Oncol 1995, 6:625-631.
37. Wagner M Expression of a truncated EGF receptor is associated with inhibition of pancreatic cancer cell growth and enhanced sensitivity to cisplatinum. Int J Cancer 1996, 68(6):782-7.
38. Matsuda K Multiple mitogenic pathways in pancreatic cancer cells are blocked by a truncated epidermal growth factor receptor. Cancer Res 2002, 62(19):5611-7.
39. Wagner M Suppression of fibroblast growth factor receptor signaling inhibits pancreatic cancer growth in vitro and in vivo. Gastroenterology 1998, 114(4):798-807.
40. Kornmann M, Arber N and Korc M Inhibition of basal and mitogen-stimulated pancreatic cancer cell growth by cyclin D1 antisense is associated with loss of tumorigenicity and potentiation of cytotoxicity to cisplatin. J Clin Invest 1998, 101(2):344-52.
41. Overholser JP Epidermal growth factor receptor blockade by antibody IMC-C225 inhibits growth of a human pancreatic cancer xenograft in nude mice. Cancer 2000, 89(1):74-82.
42. Bries C Blockade of the epidermal growth factor receptor signaling by a novel tyrosine kinase inhibitor leads to apoptosis of endothelial cells and therapy of human pancreatic cancer. Cancer Res 2000, 60(11):2926-35.
43. Ferrara N Molecular and biological properties of vascular endothelial growth factor. J Mol Med 1999, 77(7):527-43.
44. Dvorak HF Vascular permeability factor/vascular endothelial growth factor and the significance of microvascular hyperpermeability in angiogenesis. Curr Top Microbiol Immunol 1999, 237:97-132.
45. Ortega N, Hutchings H and Plouet J Signal relays in the VEGF system. Front Biosci 1999, 4:D141-52.
46. Houck KA The vascular endothelial growth factor family: identification of a fourth molecular species and characterization of alternative splicing of RNA. Mol Endocrinol 1991, 5(12):1806-14.
47. Poltorak Z VEGF145, a secreted vascular endothelial growth factor isoform that binds to extracellular matrix. J Biol Chem 1997, 272(11):1715-8.
48. Park JE, Keller GA and Ferrara N The vascular endothelial growth factor (VEGF) isoforms: differential deposition into the subepithelial extracellular matrix and bioactivity of extracellular matrix-bound VEGF. Mol Biol Cell 1999, 4(12):1317-26.
49. Nilsson S Vascular endothelial growth factor B, a novel growth factor for endothelial cells. Proc Natl Acad Sci U S A 1996, 93(6):2576-81.
50. Grimmond S Cloning and characterization of a novel human gene related to vascular endothelial growth factor. Genome Res 1996, 6(2):124-31.
51. Joukov V A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases. Embo J 1996, 15(7):1751.
52. Yamada T Molecular cloning of a novel vascular endothelial growth factor, VEGF-D. Genomics 1997, 42(3):483-8.
53. Meyer M A novel vascular endothelial growth factor encoded by Orf virus, VEGF-E, mediates angiogenesis via signalling through VEGFR-2 (Flk) but not VEGFR-1 (Flt-1) receptor tyrosine kinases. Embo J 1999, 18(2):363-74.
54. Maglione D Isolation of a human placenta cDNA coding for a protein related to the vascular permeability factor. Proc Natl Acad Sci U S A 1991, 88(20):9267-71.
55. Ferrara N Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. Nature 1996, 380(6573):439-42.
56. Carmeliet P Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature 1996, 380(6573):435-9.
57. Shibuya M Structure and function of VEGF/VEGF-receptor system involved in angiogenesis. Cell Struct Funct 2001, 26(1):25-35.
58. Veikkola T Regulation of angiogenesis via vascular endothelial growth factor receptors. Cancer Res 2000, 60(2):203-12.
59. Neufeld G Vascular endothelial growth factor (VEGF) and its receptors. Faseb J 1999, 13(1):9-22.
60. Kukk E VEGF-C receptor binding and pattern of expression with VEGFR-3 suggests a role in lymphatic vascular development. J Cell Sci 2001, 114(12):4309-18.
61. Gluzman-Poltorak Z Neuropilin-2 is a receptor for the vascular endothelial growth factor (VEGF) forms VEGF-145 and VEGF-165. J Biol Chem 2000, 275(38):29922.
62. Feng GH Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. Nature 1995, 376(6535):26-70.
63. Shalaby F Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature 1995, 376(6535):62-6.
64. Takahashi S Targeting of both mouse neuropilin-1 and neuropilin-2 genes severely impairs developmental yolk sac and embryonic angiogenesis. Proc Natl Acad Sci U S A 2002, 99(6):3657-62.
65. Hanahan D and Folkman J Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 1996, 86(3):353-64.
66. Fang J HIF-1alpha-mediated up-regulation of vascular endothelial growth factor, independent of basic fibroblast growth factor, is important in the switch to the angiogenic phenotype during early tumorigenesis. Cancer Res 2001, 61(15):5721-5.
67. Giordano FJ and RS Johnson Angiogenesis: the role of the microenvironment in flipping the switch. Curr Opin Genet Dev 2001, 11(1):35-40.
68. Udagawa T Persistence of microscopic human cancers in mice: alterations in the angiogenic balance accompanies loss of tumor dormancy. Faseb J 2002, 16(11):1361-70.
69. Folkman J What is the evidence that tumors are angiogenesis dependent? Natl Cancer Inst 1990, 82(1):4-6.
70. Folkman J Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med 1999, 5(1):27-31.
71. Santin AD Secretion of vascular endothelial growth factor in ovarian cancer. Eur J Gynaecol Oncol 1999, 20(3):177-81.
72. Plate KH Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. Nature 1992, 359(6398):845-8.
73. Goldman CK Brain edema in meningiomas is associated with increased vascular endothelial growth factor expression. Neurosurgery 1997, 40(6):1269-77.
74. Okada F Impact of oncogenes in tumor angiogenesis: mutant K-ras up-regulation of vascular endothelial growth factor/vascular permeability factor is necessary, but not sufficient for tumorigenicity of human colorectal carcinoma cells. Proc Natl Acad Sci U S A 1998, 95(7):3609-14.
Growth factor induction of the angiogenic phenotype reduces angiogenesis and growth of pancreatic cancer. J Gastrointest Surg 2002, 6(2):159-66

Kim KJ Gene therapy for pancreatic cancer using an adenovirus vector encoding soluble Flt-1 vascular endothelial growth factor receptor. Pancreas 2002, 25(2):111-21

Ogawa T Anti-tumor angiogenesis therapy using soluble receptors: enhanced inhibition of tumor growth when soluble fibroblast growth factor receptor-1 is used with soluble vascular endothelial growth factor receptor. Cancer Gene Ther 2002, 9(8):633-40

Solorzano CC Inhibition of growth and metastasis of human pancreatic cancer growing in nude mice by PTK 787/ZK22 an inhibitor of the vascular endothelial growth factor receptor tyrosine kinases. Cancer Biother Radiopharm 2002, 16(5):359-70

Gupta VK Vascular endothelial growth factor enhances endothelial cell survival and tumor radioresistance. Cancer J 2002, 8(1):47-54

Hooley JH and Bouchier-Hayes D Vascular endothelial growth factor (VEGF), a survival factor for tumour cells: implications for anti-angiogenic therapy. Bioessays 2002, 24(3):280-3

Dias S VEGF(165) promotes survival of leukemic cells by Hsp90-mediated induction of Bcl-2 expression and apoptosis inhibition. Blood 2002, 99(7):2532-40

Gerber HP VEGF regulates haematopoietic stem cell survival by an internal autocrine loop mechanism. Nature 2002, 417(6892):954-8

Tang RF Overexpression of lymphangiogenic growth factor VEG-C in human pancreatic cancer. Pancreas 2001, 22(3):285-92

Saimura M Tumor suppression through angiogenesis inhibition by SUIT-2 pancreatic cancer cells genetically engineered to secrete NK4. Clin Cancer Res 2002, 8(10):3243-9

Kleeff J Detection and localization of Mip-3alpha/Exo-Edus, a macrophage proinflammatory chemokine, and its CCR6 receptor in human pancreatic cancer. Int J Cancer 1999, 81(4):650-7

Le X Molecular regulation of constitutive expression of interleukin-8 in human pancreatic adenocarcinoma. J Interferon Cytokine Res 2000, 20(11):935-46

Shi Q Constitutive and inducible interleukin 8 expression by hypoxia and acidosis renders human pancreatic cancer cells more tumorigenic and metastatic. Clin Cancer Res 1999, 5(11):3711-21

Massague J TGF-beta signal transduction. Annu Rev Biochem 1998, 67:753-91

Rowland-Goldsmith MA Soluble type II transforming growth factor-beta receptor inhibits TGF-beta signaling in COLO-357 pancreatic cancer cells in vitro and attenuates tumor formation. Clin Cancer Res 2001, 7(9):2931-40

Rowland-Goldsmith MA MH, Matsuda K, Idezawa T, Ralli M, Ralli S and Korc M Soluble type II transforming growth factor-beta receptor attenuates expression of metastasis-associated genes and suppresses pancreatic cancer cell metastasis. Mol Cancer Therapeutics 2002, 1:161-167

Takeuchi Y Expression of plasminogen activators and their inhibitors in human pancreatic carcinoma: immunohistochemical study. Am J Gastroenterol 1993, 88(11):1928-33

Kleeff J Overexpression of Smad2 and colocalization with TGF-beta1 in human pancreatic cancer. Dig Dis Sci 1999, 44(9):1793-802

Kleeff J and Korc M Up-regulation of transforming growth factor (TGF)-beta receptors by TGF-beta in COLO-357 cells. J Biol Chem 1998, 273(13):7495-500

Yang EY and Moses HL Transforming growth factor beta 1-induced changes in cell migration, proliferation, and angiogenesis in the chicken chorioallantoic membrane. J Cell Biol 1990, 111(2):371-41

Lambert V Influence of plasminogen activator inhibitor type 1 on chorioidal neovascularization. Faseb J 2001, 15(6):1021-7

Andreasen PA, Egelund R and Petersen HH The plasminogen activation system in tumor growth, invasion, and metastasis. Cell Mol Life Sci 2000, 57(1):23-40

Munger JS Latent transforming growth factor-beta: structural features and mechanisms of activation. Kidney Int 1997, 51(5):1376-82

Page 7 of 8 (page number not for citation purposes)
124. Kojima S, Nara K and Rifkin DB Requirement for transglutaminase in the activation of latent transforming growth factor-beta in bovine endothelial cells. J Cell Biol 1993, 121(2):439-48

125. Cantero D Enhanced expression of urokinase plasminogen activator and its receptor in pancreatic carcinoma. Br J Cancer 1997, 75(3):388-95

126. Ishiwata T Altered expression of insulin-like growth factor II receptor in human pancreatic cancer. Pancreas 1997, 15(4):367-73

127. Elsasser HP Characterization of a transglutaminase expressed in human pancreatic adenocarcinoma cells. Eur J Cell Biol 1993, 61(2):321-8

128. Haroon ZA Tissue transglutaminase is expressed, active, and directly involved in rat dermal wound healing and angiogenesis. Faseb J 1999, 13(13):1787-95

129. Mishima K A peptide derived from the non-receptor-binding region of urokinase plasminogen activator inhibits glioblastoma growth and angiogenesis in vivo in combination with cisplatin. Proc Natl Acad Sci U S A 2000, 97(15):8484-9

130. Schwarte-Waldhoff I Smad4/DPC4-mediated tumor suppression through suppression of angiogenesis. Proc Natl Acad Sci U S A 2000, 97(17):9624-9

131. Liu D EGFR is a transducer of the urokinase receptor initiated signal that is required for in vivo growth of a human carcinoma. Cancer Cell 2002, 1(5):445-57

132. Bancroft CC Effects of pharmacologic antagonists of epidermal growth factor receptor, PI3K and MEK signal kinases on NF-kappaB and AP-1 activation and IL-8 and VEGF expression in human head and neck squamous cell carcinoma lines. Int J Cancer 2002, 99(4):538-48

133. Hirata A ZD1839 (Iressa) induces antiangiogenic effects through inhibition of epidermal growth factor receptor tyrosine kinase. Cancer Res 2002, 62(9):3554-60

134. Tsuzuki Y Pancreas microenvironment promotes VEGF expression and tumor growth: novel window models for pancreatic tumor angiogenesis and microcirculation. Lab Invest 2001, 81(10):1439-51

135. Teraoka H Enhanced VEGF production and decreased immunogenicity induced by TGF-beta 1 promote liver metastasis of pancreatic cancer. Br J Cancer 2001, 85(4):612-7

136. Ebert MP Reduced PTEN expression in the pancreas overexpressing transforming growth factor-beta 1. Br J Cancer 2002, 86(2):257-62

137. Wen S PTEN controls tumor-induced angiogenesis. Proc Natl Acad Sci U S A 2001, 98(8):4622-7

138. Liu CD Vascular endothelial growth factor is increased in ascites from metastatic pancreatic cancer. Int J Clin Oncol 2001, 6(2):59-65

139. Buchler P VEGF-R1 Influences the Prognosis of Pancreatic Cancer. Ann Surg 2002, 236(6):738-49

140. Baker CH, Solorzano CC and Fidler IJ Angiogenesis and cancer metastasis: antiangiogenic therapy of human pancreatic adenocarcinoma. Int J Clin Oncol 2001, 6(2):59-65

Publish with BioMed Central and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."
Sir Paul Nurse, Cancer Research UK

Your research papers will be:
- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp