The molecular basis of pancreatic cancer

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INTRODUCTION

Carcinoma of the pancreas (PC) is an aggressive disease with a poor prognosis and an overall 5-year survival of less than 1%, making it the 4th to 5th leading cause of cancer-related mortality in the Western world. Surgery remains the only treatment option with a chance for cure, whereas radiotherapy and/or chemotherapy as well as newer experimental therapeutic modalities, such as antihormonal therapy or systemic use of anti-pancreatic cancer cell monoclonal antibodies, have not led to a substantial improvement in patient survival.

In the last two decades, efforts have been made to characterize the molecular alterations that are present in PC, often with the long-term goal of improving the diagnosis and prognosis of PC patients. A number of gene mutations, including k-ras proto-oncogene mutations and p53, p16, and SMAD 4 tumor-suppressor gene mutations, are frequently present in PC. Moreover, growth factors (GFs) and their receptors (GFRs) play an important role in pancreatic tumorigenesis. In addition, clarifying the role of transcription factors (TFs), which control the initiation and extent of gene transcription, has been the focus of pancreatic cancer research. The present study reviews the molecular alterations implicated in the development of PC, and includes gene mutations, GFs, GFRs, as well as TFs that combine and give pancreatic cancer cells a major growth advantage, resulting in rapid tumor progression, resistance to chemotherapy, radiotherapy, immunotherapy, and hormone therapy, and finally poor survival.

A) GENETIC ALTERATIONS

a) Ras mutations in pancreatic cancer

The human ras family includes three proteins, termed H-ras, N-ras and K-ras, which serve in signal transduction pathways. They exist in either active or inactive states. Point mutations of K-ras constitute common alterations in human cancers, and frequently occur at codons 12, 13, or 61. K-ras point mutations at codon 12 are present in approximately 85-95% of PC, with the most frequent amino-acid substitution being a G to A transition. K-ras mutations result in constitutive activation of the protein, which subsequently signals via the ras/raf/mitogen-activated protein kinase (MAPK) cascade to enhance cell proliferation, besides other effects. K-ras mutations are already present in atypical ductal hyperplasia and in a small percentage of chronic pancreatitis (CP) lesions, suggesting that these mutations occur early in the carcinogenesis of PC.

b) p53 in pancreatic cancer

The tumor suppressor gene p53 is located on the short arm of chromosome 17 and encodes a 53-kd nuclear phosphoprotein, which functions as a transcription factor that triggers cell cycle arrest and/or apoptosis in response to DNA damage. The former is achieved in part by induction of the expression of p21WAF1, an inhibitor of cyclin-dependent kinases at the so-called G1/S cell cycle checkpoint preceding cell replication. p53 can also trigger apoptosis in different cell types through not yet completely understood mechanisms. Up-regulation of the expression of the proapoptotic Bax protein, a Bcl-2 homologue, and downregulation of cyclin A are two of the implicated mechanisms. p53 mutations are common in human cancers (40-60% of all tumors), and are

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found in 23-61% of pancreatic cancers. Overexpression of mutated p53, which is thought to be caused by enhanced stability of the mutated protein, correlates with tumor stage, tumor size, and lymph node metastasis. In addition, enhanced expression of p53 is associated with shorter survival of PC patients following tumor resection.

c) Other molecular alterations in pancreatic cancer

1. A recently discovered genetic alteration in PC is the mutation or deletion of Smad 4 (also termed Deleted in Pancreatic Cancer 4, DPC4). Smad4 mutations occur in approximately 50% of PCs. Smad 4 is involved in the signal transduction pathway of the transforming growth factor-β (TGF-β) family. Smad 4 mutations are thought to block the growth inhibitory effects of TGF-βs in PC.

2. p16, which is a cyclin-dependent kinase (CKD) inhibitor, is altered in up to 85% of PCs, resulting in cell cycle progression through Rb retinoblastoma protein phosphorylation.

3. The pro-apoptotic Bax gene is often up-regulated in PC, and enhanced expression correlates with longer survival, indicating that apoptotic pathways are of biological significance in PC.

4. Overexpression of the anti-apoptotic Bcl-2 gene occurs in approximately 50% of human PCs. The Bcl-xL anti-apoptotic gene, which is a member of the Bcl-2 family, is also overexpressed in 50% of human PCs. Bcl-xL is thought to promote tumor progression and influence survival negatively, possibly by providing PC cells with a protective effect against naturally occurring or cytotoxic-induced apoptosis.

5. TNF-alpha and Fas ligand-mediated apoptosis is blocked in PC probably by overexpression of inhibitory mediators or decreased expression of essential signal components of this pathway in PC cells, which disrupt the aforementioned apoptotic pathway.

6. The expression of the Id-2 gene, which acts as a dominant negative transcription factor, is increased in PC, and this increase is associated with decreased differentiation and enhanced proliferation of PC cells.

7. KAI1, which is a membrane glycoprotein implicated in cell-cell interaction, invasiveness and metastasis, is overexpressed in the early stage of PC, while the levels of expression are lower in advanced stages and metastatic PC, suggesting a tumor suppressor function of this gene in PC.

8. Fragile histidine triad gene (FHIT), which encodes for a hydrolase, is found on chromosome 3 and is mutated in 70% of PC cell lines.

B) GROWTH FACTORS AND GROWTH FACTOR RECEPTORS

Growth factors are produced by many different cell types and exert their pleiotropic effects via autocrine and/or paracrine mechanisms. They are also involved in carcinogenesis, where they influence a variety of functions, including cell proliferation, cell invasion and metastasis, angiogenesis, local immune functions, and extracellular matrix formation.

a) The transforming growth factor β (TGFβ) superfamily and its receptors

The mammalian TGF-β superfamily of pleiotropic GFs includes the TGF-β family itself (TGF-β1, -β2 and -β3), the activin/inhibin family, the bone morphogenic protein (BMP) family, the Vg-1 family and Müllerian inhibitory substance. These GFs are usually synthesized as precursors that undergo proteolytic cleavage to yield biologically active proteins.

TGF-βs signal through the cell surface TGF-β receptors, with serine-threonine-kinase activity. They comprise three types: the type I (TβRI), type II (TβRII) and type III (TβRIII) receptors. TβRI and TβRII are important for signal transduction, while cell surface expression of TβRIII modulates the binding of TGF-βs to TβRII. PCs overexpress all three TGF-β isoforms, and the overexpression of any of these isoforms is associated with a worse prognosis following tumor resection. Furthermore, TβRII is also expressed at increased levels in the human PC cells in comparison with the normal pancreas. However, PC cells are usually resistant to the growth inhibitory effects of TGF-βs. This might be due to several genetic and epigenetic alterations, such as low levels of TβRII or mutations of the Smad 4 gene, a member of the Smad family of recently identified intracellular signal transducers of the TGF-β superfamily. Smad 4, which binds to all pathway-specific Smads, is thought to be essential for the transcriptional activation of TGF-β target genes. Smad6 and Smad7, which antagonize the function of pathway-specific Smads, are overexpressed in PCs. In PC cells, this leads to resistance of TGF-β-mediated growth inhibition while still allowing for the induction of PAI-1, which might function to enhance tumor invasion and metastasis.
mor cell-derived TGF-β may act in a paracrine manner to enhance angiogenesis and suppress cancer-directed immune mechanisms. TGF-βs may also act to increase the expression of adhesion molecules and extracellular matrix components including fibronectin, collagen and laminin, thereby enhancing the metastatic potential of cancer cells.

PCs also markedly overexpress the activin/inhibin βA subunit and to a lesser extent the βB subunit, as well as BMP2 and its type Ia and type II receptors.54

b) The epidermal growth factor (EGF) superfamily and its receptors

Epidermal growth factor (EGF), a maintenance factor for the continuous renewal of the epithelial cell population, belongs to a family with many members besides EGF itself. These include transforming growth factor-alpha (TGF-α), heparin-binding EGF-like growth factor (HB-EGF), amphiregulin, betacellulin, epiregulin, neuregulins and cripto. All these EGF-related growth factors share amino-acid homology. The EGF receptor is a 170 kDa glycoprotein found on the cell surface of a variety of cell types and characterized by its ligand-dependent tyrosine kinase activity. The EGF receptor, also known as human EGF receptor 1 (HER 1), is closely related to several other receptors such as HER 2 (c-erbB2), HER 3 (c-erbB3), and HER 4 (c-erbB4). The activated receptors transmit signals through a variety of intracellular substrates, depending on the cell type, the ligand, and the participating EGF receptors. A number of intracellular signaling proteins, such as phosphatidylinositol-3 (PI3) kinase, phospholipase C-γ, src and GRB2, bind directly to the phosphorylated EGF receptor. PI3 kinase has been found to play a role in cell invasion.72 src and GBR2 link the EGF receptor to the SOS/ras/raf-1/MAP kinase pathway, which results in phosphorylation of jun and fos nuclear protooncogenes, thereby ultimately leading to cell proliferation.

Concomitant overexpression of EGF, TGF-α, EGF receptor and HER3 has been demonstrated in human PC in several studies, indicating that autocrine and paracrine mechanisms of the receptor-ligand system play a crucial role in the pathogenesis of PC. In support of this hypothesis, transgenic mice overexpressing TGF-α in the exocrine pancreas show dysplastic changes of the pancreas, resembling those seen in human PC, and display progressively increased EGF receptor levels during the transition from acinar to duct-like transformed cells.50 Furthermore, blockage of EGF receptor-dependent signaling pathways in PC cells leads to decreased anchor-independent growth of these cancer cells.87

c) The fibroblast growth factor (FGF) family and its receptors

Fibroblast GFs (FGFs) are a family of heparin-binding polypeptide GFs that activate transmembrane tyrosine kinase receptors. They are involved in mitogenesis, cell differentiation and angiogenesis, and presently consist of at least 14 members.59-63 Acidic FGF (FGF 1 or aFGF) and basic FGF (FGF 2 or bFGF), the prototypes of this GF family, and FGF 5, and keratinocyte GF (FGF 7 or KGF), are overexpressed in PC.52,54-55 Signaling by the FGFs is mediated by a dual-receptor system consisting of four high-affinity transmembrane tyrosine-kinase FGF receptors (FGFRs) that function as signaling molecules to transmit the effects of FGFs, as well as by low-affinity heparansulfate-proteoglycans (HSPGs) that are devoid of signaling capabilities but enhance ligand presentation to FGFRs. FGF-1 and FGF-2 and its splice variant keratinocyte growth factor receptor (KGF), are also expressed at high levels in PC cells in vivo. Glypican-1, a GPI-anchored protein, which seems to be the most important co-receptor for heparin-binding GFs, is overexpressed in a large proportion of pancreatic cancers, and its expression occurs predominantly in the PC cells and in the fibroblasts surrounding the tumor mass.

Experimental evidence for the importance of FGF in PC is provided by studies demonstrating that blockage of FGF receptor-dependent pathways can lead to decreased tumor growth in vivo in nude mice.

d) Nerve growth factor (NGF) and its receptors

Nerve growth factor (NGF), a neurotrophic protein involved in maintenance and differentiation of a variety of neural cell types, has recently been suggested as stimulating tumor growth, cancer cell invasion, and formation of metastases.101-103 TrkA is the high-affinity receptor for NGF and is an essential component in mediating NGF signals. NGF and TrkA mRNA levels are significantly increased in PC tissues compared with the normal pancreas.105 Tumors with high TGF and TrkA expression levels exhibit more frequent perineural invasion. Perineural invasion extending to the extrapancreatic nerve plexus is a histopathologic characteristic which leads to retropancreatic tumor extension, precludes curative resection, promotes local recurrence and finally influences the prognosis of the patient negatively. However, the mechanisms that contribute to invasion of pancreatic nerves and to the spread of cancer cells along nerves are as yet poorly understood. Furthermore, increased NGF and TrkA expression levels have also been
associated with a higher degree of pain in patients with PC.\textsuperscript{105}

e) The platelet-derived growth factor (PDGF) family and its receptors

Platelet-derived growth factors (PDGF) consist of A and B chains, which are linked via disulfide bonds, forming three isoforms: PDGF-AA, PDGF-AB and PDGF-BB.\textsuperscript{106} PDGF stimulates growth and chemotaxis of fibroblasts, smooth muscle cells and other cell types. It also regulates growth and differentiation of fetal cells and stimulates wound healing in adults. The PDGF A and B chains and their receptors are highly expressed in PC tissues compared with the normal pancreas,\textsuperscript{110} and are thought to be regulated, at least in part, by TGF-\textbeta1.

f) The insulin-like growth factors (IGFs) and their receptors

The IGF family includes insulin-like GFs type I and II (IGF-I and IGF-II), which are structurally related to pro-insulin. IGFs bind to IGF receptors (IGF-I receptor and IGF-II/M-6-P receptor), IGF-binding proteins (IGFBPs; IGFBP-1 to –6) and IGFBP-related proteins (IGFBP-rP-1 to –9).\textsuperscript{111-113} They are potent mitogens whose action is determined by the availability of free IGF to interact with the IGF receptors. The latter are regulated by the rate between IGF production, IGF clearance, and the degree of IGF binding to IGFBPs.\textsuperscript{111} Overexpression of IGF-I and IGF-I receptor has been demonstrated in human PC tissues,\textsuperscript{114} in which the IGF-I/IGF-I receptor system is believed to act in an autocrine way to control local cancer cell growth.\textsuperscript{115} Furthermore, IGF-I stimulates the growth of cultured PC cells, and this enhanced growth is inhibited by alpha-IR3, a specific anti-IGF-I antibody, and by IGF antisense oligonucleotides.\textsuperscript{114}

C) TRANSCRIPTION FACTORS (TFs)

TFs are nuclear proteins which play a role in the initiation and extent of gene transcription, by recognizing specific DNA sequences at the promoter site of the gene. They comprise two subgroups: the basal machinery or general transcription factor group, required for the initiation of transcription of most genes, and the gene- or cell-type-specific TF group. Transcriptional deregulation is an important event in the neoplastic process,\textsuperscript{116} and deregulated TF expression has been reported in numerous cancers.\textsuperscript{117-118} In PC, a number of TFs are implicated in the ras-mediated activation of transcription, including AP-1 (c-jun, c-fos), SRF (p67SRE, p62TCF), ETS (Elk 1, pointed 1 and 2), NF-IL-6, c-myc, and NF-KB/Rel.\textsuperscript{119} c-myc, c-jun and c-fos function as oncogenic TFs, which are involved in tissue-specific gene expression.\textsuperscript{120-121} c-fos/c-jun heterodimers can bind to AP1 recognition sites, thus enhancing gene transcription, suggesting a crucial role in the development and progression of PC.\textsuperscript{122-124} Finally, p53, as mentioned above, acts as a transcription factor that regulates cell cycle and programmed cell death (apoptosis), through induction of a set of genes with negative effects on cell growth.\textsuperscript{125-126}

CONCLUSION

Modern molecular biology techniques have contributed significantly to a better understanding of the pathophysiological changes in PC. As mentioned in this paper, molecular alterations of a variety of genes, TFs, GFs and GFRs have been demonstrated in this disease, indicating that these alterations may result in a major growth advantage of PC cells, which clinically results in fast tumor progression. A better understanding of the molecular alterations of PC will ultimately lead to improvements in diagnostic and therapeutic strategies and subsequently to a better prognosis for patients afflicted with this deadly disease.

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