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Molecular prognostic markers in pancreatic cancer

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Abstract Pancreatic cancer has a very poor prognosis and is a common cause of cancer death in the Western world. Certain genetic alterations may be important in the prognosis of pancreatic cancer. Activation mutations in the K-*ras* oncogene occur in around 90% of pancreatic cancers, and the overexpression of growth factors epidermal growth factor (EGF), transforming growth factor (TGF)α, TGFβs 1–3, acidic fibroblast growth factor (aFGF), basic FGF (bFGF), and growth factor receptors c-erbB-2 and -3 and TGFRβs 1–3 is common. High mutation levels of cell cycle control genes such as *p53*, *p16*, *p21*, *SMAD4*, and cyclin D1 are found, and there is abnormal expression of apoptotic genes, such as *bcl-2*, *bcl-XL*, and *bax*. The expression of several of these growth factors and their receptors has been found to be associated with poorly differentiated tumors of an advanced stage and decreased survival. However, the inactivation and loss of expression of *p16*, *p53*, and *p21*, and the expression of several apoptotic genes, such as *bax* and *bcl-2*, have not been found to be of any prognostic significance. The expression of wild type *p53*, however, may predict responsiveness to chemotherapy. TGFβ1 expression has been shown to be associated with longer survival in patients with pancreatic cancer. Two studies (including our own) have found *bcl-XL* expression to be significantly associated with poor survival. These and newer molecular markers may prove to be important in the choice of future therapies for pancreatic cancer.

Key words Pancreatic cancer · Molecular marker · Prognosis

Introduction

Pancreatic cancer is a common cause of cancer-related death in the Western world, with an overall 5-year survival of 0.4%.[1] This aggressive tumor presents at a late stage, and potentially curative surgical resection is possible in only about 10%–15% of patients.[2,3] Moreover, currently available radiotherapy and chemotherapy regimens have not shown improved survival in advanced disease; their role in adjuvant therapy is currently being assessed.[4,5] Prognostic markers may identify subgroups of patients who may benefit better from aggressive therapy given in an attempt to improve survival. Clinical parameters that are significant in predicting patient outcome are tumor grade, stage, resection margin status, and tumor size; patients with negative resection margins and stage I–II disease demonstrate superior survival rates.[6–9] Current staging systems in use include the International Union Against Cancer (UICC) TNM classification in the West[10] and the Japan Pancreas Society (JPS) system in Japan,[11] but unfortunately, they are not exactly comparable. This causes difficulties, because of "staging system" migration, when survival data are compared.

The absence of disseminated tumor cells both in the peritoneal cavity and in the bone marrow was found to be associated with significantly longer 5-year survival rates.[12] The serum concentrations of tumor markers such as CA 242, CA 19.9, and MUC 1 may have some prognostic significance in advanced disease, but not in patients with small tumors.[13–15] In pancreatic cancer there is overexpression of growth factors and growth factor receptors, including epidermal growth factor (EGF), transforming growth factor (TGF)α, EGF receptor (EGFR), amphiregulin, c-erbBs 2–4, acidic fibroblast growth factor (aFGF), basic (b)FGF, FGFR, TGFβs1–3, and TGFβRs. Inactivation of the tumor suppressor genes (TS) *p53*, *p16*, and *SMAD4*,[16] and activation of dominant oncogenes K-*ras* and cyclin D1 all play a significant role in tumorigenesis. The possible use of these molecular markers for prognosis in pancreatic cancer has been the subject of extensive work in our laboratory and others.

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Growth factors and their receptors

Although pancreatic carcinoma demonstrates abnormal expression of numerous growth factors and their receptors, which may contribute to pancreatic tumor cell growth, some of these changes also occur in chronic pancreatitis. The pattern and level of expression of these receptor-ligand systems accounts for the different disease processes, but it is noteworthy that there is at least a sixfold increased incidence of pancreatic cancer in patients with chronic pancreatitis.

Epidermal growth factor receptor (EGFR) family and its ligands

This family of type 1 growth factor receptors comprises four transmembrane tyrosine kinases: the epidermal growth factor receptor (EGFR) and c-erbBs-2, 3, and 4.[17] They share significant sequence homology with one another and are all frequently overexpressed in human pancreatic cancer. EGFR is activated by a family of peptide ligands that includes EGF, TGFα, heparin–binding EGF–like growth factor, betacellulin, and amphiregulin.[18]

EGF is a polypeptide of 53 amino acids that stimulates proliferation and differentiation of a wide variety of cell types through the EGFR.[19] The EGFR is encoded by the *c-erbB-1* protooncogene and is a transmembrane growth factor receptor with tyrosine kinase activity. In the normal pancreas, EGFR is expressed only in the islets of Langerhans. The *EGFR* gene, however, is overexpressed in 95% of ductal adenocarcinomas and human pancreatic cell lines, because of an increase in gene transcription.[20] EGFR binds EGF and TGFα with high affinity, and both of these ligands are overexpressed in pancreatic cancer.[21] The overexpression of EGFR has been shown to increase the production of EGF and TGFα, promoting autocrine and paracrine loops that promote cell proliferation.[21] TGFα is expressed at low levels in the ductal epithelium of the normal human pancreas, but it is overexpressed at high levels in 95% of ductal adenocarcinomas.[20] While EGF is not detectable in the normal pancreas, it is found in 12% of pancreatic cancers.[20] Several studies have shown that high levels of EGFR, EGF, and/or TGFα were correlated with reduced patient survival.[22,23] Shorter postoperative survival was also demonstrated with the overexpression of amphiregulin and EGFR.[24]

The *c-erbB-2* gene encodes a 185-kDa transmembrane glycoprotein with tyrosine kinase activity,[25] and acts as a receptor for a class of ligands that includes the heregulins, gp30, and NEU-differentiation factor (NDF), but the full contribution of *c-erbB-2* to growth stimulation in pancreatic cancer remains unknown. *c-erbB-2* is overexpressed in bladder, breast, esophageal, and gastric cancer, where it appears to have a role in lymph node metastases.[26,27] In invasive ductal adenocarcinomas of the pancreas and ampullary tumors, *c-erbB-2* is overexpressed in 20%, and this is usually caused by *c-erbB-2* gene amplification.[28] One study showed a survival advantage for those patients with tumors that did not overexpress *c-erbB-2*,[29] and another study correlated survival with serum levels of *c-erbB-2*.[30] But other studies, including our own, with larger patient numbers did not reveal any prognostic significance for *c-erbB-2* expression.[31]

The *c-erbB-3* gene encodes a Mr 180000 transmembrane polypeptide that shares close structural homology with the EGF receptor and *c-erbB-2*, but it has no known natural ligands. In the normal pancreas, *c-erbB-3* is seen only in the islets of Langerhans, but has been detected in up to 90% of pancreatic and ampullary cancers.[32,33] Freiss et al.[34] found that *c-erbB-3* overexpression correlated with decreased patient survival. In our own series,[31] we found overexpression in 54% of tumors but found no correlation with survival.

c-erbB-4 is a 180-kDa transmembrane tyrosine kinase whose extracellular domain is similar to that of *c-erbB-3*. The cytoplasmic kinase domain exhibits a high degree of homology with *EGFR* and *c-erbB-2*.[35] The heregulins and betacellulin can all activate *c-erbB-4*.[36,37] *c-erbB-4* is predominantly expressed in normal skeletal muscle, heart, pituitary gland, brain, and cerebellum, as well as being expressed in breast cancer cell lines. A recent study looked in detail at *c-erbB-4* expression in pancreatic cancer, using quantitative reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemistry. Survival analysis did not show any significant difference between patients with *c-erbB-4*-positive tumors and those with negative tumors.[38]

TGFβs and TGFβ receptors

Transforming growth factor-βs are a family of cytokines that actively influence cell division, cell death, and cellular differentiation. They bind to specific cell surface receptors that are able to downregulate transcription factors, decrease phosphorylation of target proteins, and inactivate cell-cycle regulatory enzymes. The end targets may be the G1 cyclins and cyclin-dependent kinases. The presence of mutant *p53* has been correlated with the loss of TGFβ-1 responsiveness in malignant epithelial cell lines. TGFβ-1 may also induce the universal cyclin inhibitor *p21* by a *p53*-independent pathway.[39,40] The three isoforms TGFβ-1–3 have been shown to be overexpressed in pancreatic cancer. Survival data from 60 patients with pancreatic cancer showed significantly decreased survival time with the overexpression of these isoforms.[41,42] In a more recent study, however, TGFβ-1 which was expressed in 31% of

cancers, was significantly associated with increased survival.[43] These contradictory findings deserve further investigation. The TGFβ receptors 1–3 are all found in normal pancreas, but in cancer there is overexpression of TGFβ-2 receptor only.[44]

Fibroblast growth factors

Fibroblast growth factors (FGFs) belong to a large group of closely homologous polypeptide growth factors which includes the two main members, acidic FGF (aFGF) and basic FGF (bFGF). These growth factors are highly abundant in the basement membrane and extracellular matrix of a variety of tissues. FGFs influence cell differentiation, tissue homeostasis, tissue regeneration and repair, cell migration, and angiogenesis. Binding to specific transmembrane receptors (which have intracellular tyrosine kinase activity) is dependent on the presence of heparin sulfate proteoglycans (on the cell surface or in the extracellular matrix) for which they have a high affinity. Both aFGF and bFGF are overexpressed in pancreatic cancer tissue at the mRNA and protein levels.[45,46] The expression of bFGF and FGF receptor (R), but not aFGF, is associated with poor prognosis[45,47] (Table 1[22,23,29,31,34,38,41,43,45,47–49]).

Tumor suppressor genes

Most tumor suppressor genes have pivotal roles in cell cycle control and apoptosis. Loss of function can be associated with uncontrolled cell growth and

Table 1. Prognostic significance of growth factors and growth factor receptors in pancreatic cancer

Study	Year	No. of patients	Factor	Method	Expression	Survival		*P*
						Median (months)	5-Year (%)	
Yamanaka et al.[22]	1993	87	EGF	IHC	+	—	—	
					—	—	—	<0.05
Uegaki et al.[23]	1997	86	EGF	IHC	+	21	—	
					—	25	—	NS
			EGFR		+	23	—	
					—	25	—	NS
Dong et al.[48]	1998	57	EGF + R	IHC	+	10	—	
					—	17	—	<0.002
Gansauge et al.[49]	1998	82	EGF + R	IHC	+	—	—	
					—	—	—	NS
Lei et al.[29]	1995	21	c-erbB-2	IHC	+	7	—	
					—	19	—	<0.01
Friess et al.[34]	1995	58	c-erbB-3	IHC	+	9	—	
					—	13	—	<0.04
Kawesha et al.[31]	1997	142	c-erbB-2	IHC	+	8	—	
					—	9	—	>0.7
			c-erbB-3	IHC	+	9	—	
					—	8	—	>0.7
Graber et al.[38]	1999	75	c-erbB-4	IHC	+	—	—	
					—	—	—	NS
Yamanaka et al.[45]	1993	78	aFGF	IHC	+	11	—	
					—	13	—	NS
			bFGF		+	9	—	
					—	16	—	<0.001
Ohta et al.[47]	1995	32	bFGF	IHC	+	8	—	
					—	9	—	NS
			FGFR		+	6	—	
					—	—	—	<0.01
Friess et al.[41]	1993	60	TGFβ-1	IHC	+	9	—	
					—	12	—	<0.05
			TGFβ-2		+	7	—	
					—	13	—	<0.001
			TGFβ-3		+	7	—	
					—	12	—	<0.01
Coppola et al.[43]	1998	42	TGFβ-1	IHC	+	13	23	
					—	5	4	<0.05

EGF, Epidermal growth factor; EGFR, epidermal growth factor receptor; IHC, immunohistochemistry; aFGF, acidic fibroblast growth factor; bFGF, basic fibroblast growth factor; FGFR, fibroblast growth factor receptor; TGFβ, transforming growth factor beta

proliferation, decreased apoptosis, and malignant transformation.

p53

The *p53* gene encodes a 53-kDa nuclear phosphoprotein consisting of 393 amino acids. Under normal conditions, the p53 protein is found in the cell nucleus. *p53* controls the entry of cells into the growth cycle at the G1/S boundary and has a critical role in initiating the repair of damaged DNA before it is replicated.[50] The inability to effect DNA repair with accuracy may result in *p53*-dependent apoptosis. The *p53* gene is mutated in over 50% of human cancers.[51,52] In pancreatic cancer it is inactivated in approximately 65% of cases by a missense point mutation; loss of the remaining normal allele commonly occurs in pancreatic cancer cell lines, although this occurs less frequently in primary tumors.[53] In contrast to ductal adenocarcinomas, only 33% of intraductal papillary adenocarcinomas, and no mucinous and adenosquamous pancreatic carcinomas, exhibit positive nuclear staining for p53.[54,55] The majority of *p53* mutations are found in exons 5–8, which contain the most highly conserved regions. Point mutations result in the production of mutant p53 proteins, which are more stable than the wild-type protein and accumulate within the nucleus. *p53* gene mutations probably occur relatively early in the process of pancreatic carcinogenesis.[55] Lundin et al.[56] found that *p53* mutations did not correlate with tumor stage, histology, age, sex, or survival. Our study, in which 35% of cancers were positive by immunostaining, demonstrated no correlation with patient survival[31] (Table 2).

When cells are exposed to either radiation or cytotoxic agents, the levels of wild-type p53 protein rise 5 to 60-fold (because of increased stability of the protein, rather than increased protein synthesis). It follows that tumors with functioning p53 may carry a better prognosis than those expressing the mutant protein, when exposed to radiation or DNA-damaging agents. Two studies investigated 58 patients, of whom 28 had surgery alone and the other 30 had adjuvant chemotherapy. Patients with p53 positive tumours (shown by immunostaining) who had chemotherapy had significantly better survival times than those who did not.[57] Patients with combined p53 positive and p21 negative tumors had significantly poorer survival and those in this group who had chemotherapy had a nonsignificant trend for improved survival.[58]

p16

The *p16* tumor suppressor gene encodes a 16-kDa protein[59] that plays a key role in controlling the G1 checkpoint (or the restriction point, R) of the cell cycle (Fig. 1). The retinoblastoma gene product (pRb) is an active transcriptional repressor when bound to transcription factors such as the E2F family. Inactivation of pRb by phosphorylation (mediated by the complex formed by CDK4,6 and cyclin D) causes release of E2F and subsequent transcription of genes important for DNA synthesis.[60] The p16 protein prevents the association of CDK4 and 6 with cyclin D and the subsequent phosphorylation of pRb. This growth suppression by p16 requires functional retinoblastoma protein.[61] Loss of p16 expression occurs in up to 85% of pancreatic cancer cell lines and xenografts,[62] caused by homozygous or heterozygous deletion, and hypermethylation.[63] A study of 32 patients with pancreatic cancer found a significant correlation between p16 negativity and poor prognosis.[64] In our study of 142 patients, however, the p16 negativity rate on immunohistochemical staining was 80%, but there was no correlation with survival.[31]

p21^{WAF1}

p21^{WAF1}, a cyclin-dependent kinase inhibitor, is a downstream target and effector of p53. The 21-kDa product of the *WAF1* gene forms part of a quaternary complex, along with cyclin/CDKs and the proliferating cell nuclear antigen (PCNA) in normal cells, but not in transformed cells, and is a universal inhibitor of CDK activity.[65] One consequence of p21 binding to and inhibiting CDKs is to prevent CDK-dependent phos-

Table 2. Tumor suppressor genes in pancreatic cancer

Study	Year	No. of patients	Gene	Method	Expression	Median survival (months)	*P*
Lundin et al.[56]	1996	133	*p53*	IHC	+	9	
					—	12	NS
Kawesha et al.[31]	1997	142	*p53*	IHC	+	10	
					—	8	>0.2
			p16	IHC	+	10	
					—	11	>0.2
			p21	IHC	+	11	
					—	8	>0.6

IHC, Immunohistochemistry

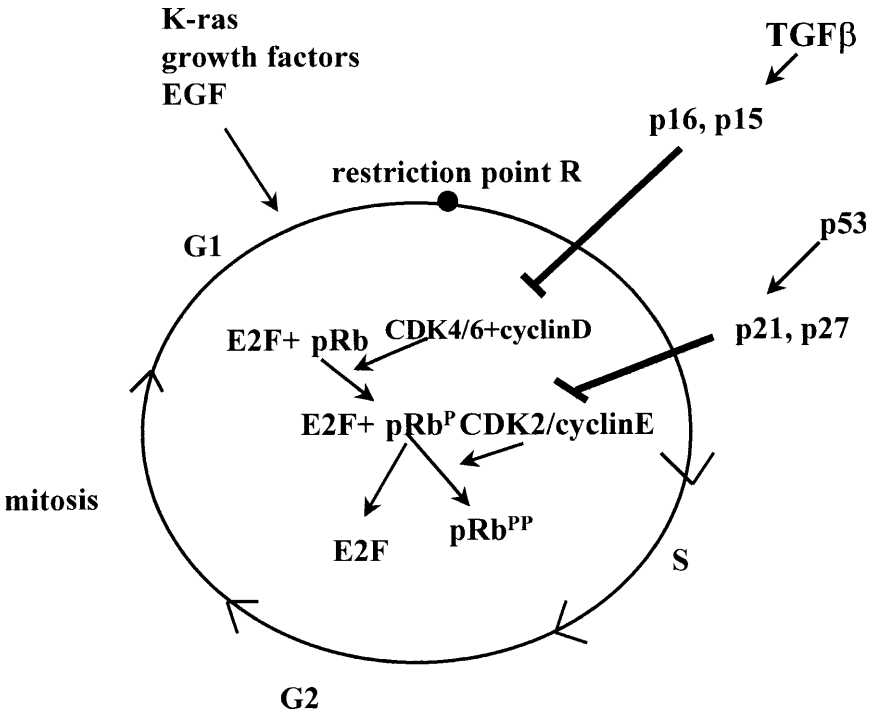

Fig. 1. The cell cycle. The retinoblastoma gene product, pRb, is central to G1 checkpoint control.[60] *EGF*, Epidermal growth factor; *TGFβ*, transforming growth factor β

phorylation and subsequent inactivation of the Rb protein, which is essential for cell cycle progression at both the G1 and G2 checkpoints. The negative regulatory action of $p21^{WAF1}$ on the cell cycle permits sufficient time for repair to extensive DNA damage to be completed. Functional *p21* is essential for *p53*-mediated G1 arrest due to WAF1 inhibition of both CDK activity and PCNA-dependent DNA replication.[65] Thus, *p21* overexpression causes growth suppression, consistent with its role as an inhibitor of CDKs and as a tumor suppressor gene. Induced expression of $p21^{WAF1}$ by *p53* directly, or by *p53*-independent mechanisms, results in as much as a 20- to 100-fold increase, depending on the cell type and mode of induction. Kinetic analyses of *p53*-dependent induction indicate that $p21^{WAF1}$ expression begins to rise coincident with the accumulation of p53 in response to DNA damage. Failure to show this rise is associated with failure of G1 arrest and with inappropriate onset of apoptosis.[66] Nevertheless, all studies our own, included, have not shown any prognostic value in p21 expression, as demonstrated with immunohistochemistry.[30,43,67,68]

Oncogenes

K-ras

The *ras* family of protooncogenes, which includes H-*ras*, N-*ras*, and K-*ras*, encodes proteins with GTP-ase activity, which, thereby, function as molecular switches in signal transduction. The K-*ras* gene encodes for a 2.0-kb transcript that is highly conserved across species and is translated into the p21-ras protein. Growth and differentiation signals from activated cell membrane receptors are transduced by K-*ras* to activate protein kinases, including cyclin kinases. Point mutations in codons 12, 13, and 61 of K-*ras* result in the expression of altered protein products that are capable of transforming cells into a malignant phenotype. The K-*ras* gene is mutated in 75% to 90% of pancreatic cancers.[69,70] The mutations present in pancreatic cancer are almost exclusively at codon 12, and only a minority of tumours demonstrate a mutation at codon 13. The spectrum of mutations in pancreatic cancer includes the aspartic acid mutation (GAT), valine (GTT), arginine (CGT), and cysteine (TGT).[71] The presence or the type of K-*ras* mutations in pancreatic tumors has not been shown to be associated with patient survival[72,73] (Table 3).

Cyclin D1

Cyclin D1 is a cell cycle regulator that may act as an oncoprotein. It forms part of the enzyme complexes that are active in G1 phase of the cell cycle and which inactivate pRb by phosphorylation. These enzyme complexes (CDKs) contain two components, a regulatory subunit — the cyclin — and a catalytic subunit — the cyclin-dependent kinase (cdk).[74] The CDKs can be activated by cdk binding cyclin and by phosphorylation of a conserved threonine by CDK-activating kinase (CAK).

Table 3. Oncogenes in pancreatic cancer, and correlation to patient survival

Study	Year	No. of patients	Gene	Method	Expression	Median survival (months)	*P*
Hruban et al.[72]	1993	82	K-*ras*	RFLP	+	14	
					—	10	>0.5
Kawesha et al.[31]	1997	142	K-*ras*	SSCP	+		
					—		NS
			Cyclin-D	IHC	+	9	
					—	8	>0.4
Gansauge et al.[49]	1998	82	Cyclin-D	IHC	+	10	
					—	18	<0.01

RFLP, Restriction fragment length polymorphism; SSCP, single-strand conformational polymorphism; IHC, immunohistochemistry; NS, not significant

Inactivation of the active cdk-cyclin complex can occur by binding to CDK inhibitory subunits (CKIs).[75] There are two classes of CKIs: the INK4 kinase inhibitors, p15, p16, p18, and p19, which specifically regulate complexes of cyclin D1, D2, and D3 with CDK4 and 6; and a second group, p21, p27, and p57, which inhibit all G1 cyclin/cdk complexes. The primary regulator of CDK activity is the cyclin subunit, whose levels oscillate during the cell cycle. The G1 cyclin complexes consist of D-type cyclins (D1, D2, and D3) complexed with either cdk4 or 6, cyclin E complexed with cdk2, and cyclin A complexed with cdk2. The appearance of D-type cyclins is tightly linked to growth factor exposure, and their downstream cell cycle effects are caused by the inactivation of pRb by phosphorylation. Cyclin D1 has been implicated in the pathogenesis of a number of cancers, including esophageal, lung, head and neck, bladder, and pancreatic, as well as sarcomas. Overexpression of cyclin D1 leads to constitutive phosphorylation of pRb and, thus, deregulated (and increased) E2F activity. Also, activation mutations of the cdk4 have been identified in certain tumors, such as melanoma.[76,77] Thus, both cyclin D1 and cdk4 can act as oncoproteins by inactivating pRb. A study of 82 pancreatic cancers demonstrated overexpression (by immunostaining) of cyclin D1 in 65% of tumors, and this was associated with shorter survival for these patients, but not independently of tumor stage and grade.[49] In our series of 142 pancreatic cancers, we found no prognostic significance associated with the expression of cyclin D1.[31]

Apoptotic factors

Apoptosis or programmed cell death is a central regulator of homeostasis in normal tissue. Damaged cells are eliminated without an immune response, and apoptosis balances cell proliferation under normal physiological conditions. There are numerous apoptotic pathways. The *bcl-2* family of apoptotic genes includes *bcl*-2, *bcl-x*, *bax*, and *bak*, plus others, and the majority have four conserved domains (Fig. 2[78]). The first two domains are important for homo- and heterodimerization, and the fourth is important for normal function. *bcl-2* is an anti-apoptotic factor and sometimes is referred to as a co-operating oncogene. By itself it is unable to transform cells, but, when activated in the presence of other oncogenes, *bcl-2* is vital to malignant transformation. *Bcl-x* is a *bcl-2*-related gene and exists as two subforms; *bcl-xL* is the longer form and functions as an apoptotic inhibitor;[79] *bcl-xS* is the shorter form and functions as an apoptosis promoter. *bax* is a promoter of apoptosis and is has been shown that the ratio between *bax* and *bcl-2* can be important in determining cell survival. An excess of *bax* homodimers promotes cell death, whereas an excess of *bcl-2* homodimers will inhibit apoptosis.[80] Several recent studies have looked at these factors in pancreatic cancer (Table 4). In a study of 60 patients, tumor expression of bax immunostaining was associated with significantly longer survival compared with that in patients who had negatively staining tumors,[81] but this was not confirmed in our own study.[82] *bcl-2* also has no prognostic significance,[81] but several studies have shown that the expression of *bcl-xL* is significantly associated with poor survival.[82–84] This is suprising, because *bcl-xL* inhibits apoptosis, and this would suggest reduced anti-apoptosis by heterodimerization.

Tumor angiogenesis

Angiogenesis is essential for tumor growth and metastasis. Angiogenic factors include aFGF and bFGF, which stimulate the locomotion and proliferation of endothelial cells, and TGFα, which has an effect on endothelial cell proliferation. These and other angiogenic factors (Table 5)[85–88] can be produced by the tumor, as well as by endothelial and stromal cells.

Angiogenin

Angiogenin (ANG) is a Mr 14100 polypeptide that is an inducer of vascularization. ANG may interact with en-

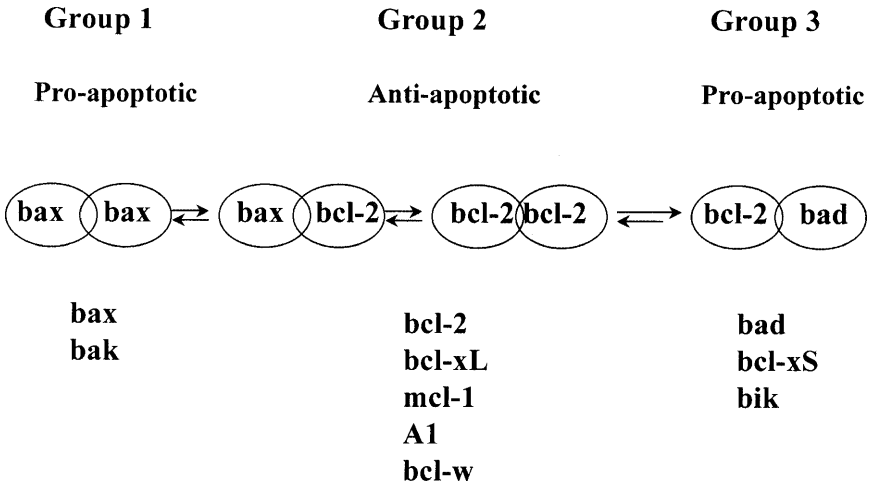

Fig. 2. Members of the *bcl-2* family and their role in apoptosis (after reference 78, personal communication)

Table 4. Prognostic value of the *bcl-2* family in pancreatic cancer

Study	Year	No. of patients	Factor	Method	Expression	Median survival (months)	*P*
Friess et al.[81]	1998	60	*bax*	IHC	+	12	
					—	5	<0.04
			bcl-2		+	—	
					—	—	NS
Friess et al.[82]	1998	74	*bcl-xL*	In situ	+	5	
					—	12	<0.05
Evans et al.[83]	1998	24	*bax*	IHC	+	12	
					—	9	NS
			bcl-xL		+	6	
					—	20	<0.002

IHC, Immunochemistry; in situ, in situ hybridization

Table 5. Angiogenic and stromal factors in pancreatic cancer

						Survival		
Study	Year	No. of patients	Factor	Method	Expression	Median (months)	1 Year (%)	*P*
Shimoyama et al.[85]	1996	37	ANG	In situ	+	10	33	
					—	13	75	<0.05
Fujimoto et al.[86]	1998	50	VEGF	IHC	+	12	—	
					—	12	—	>0.04
			PD-ECGF		+	8	—	
					—	13	—	<0.04
Cantero et al.[87]	1997	30	uPA + uPAR	IHC	+	9	—	
					—	18	—	<0.006
Kuniyasu et al.[88]	1999	22	MMP2/9	In situ	High	18	—	
			Ecad		Low	—	—	<0.0002

ANG, Angiogenin; VEGF, vascular endothelial growth factor; PD-ECGF, platelet-derived endothelial cell growth factor; uPA, urokinase plasminogen activator; uPAR, urokinase plasminogen activator receptor; MMP, matrix metalloproteinase; Ecad, E-cadherin; IHC, immunohistochemistry; in situ, in situ hybridization

dothelial cells via a cell surface receptor and extracellular matrix (ECM) molecules such as proteoglycans.[89] ANG has been shown to bind to actin on the endothelial cell surface, and this complex may lead to the activation of several protease cascades. In patients with pancreatic cancer, high levels of mRNA expression and high levels of serum ANG were significantly associated with poorer survival.[85]

Vascular endothelial growth factor (VEGF) and platelet-derived endothelial cell growth factor (PD-ECGF)

VEGF is a very potent and selective endothelial cell mitogen that has been shown to be associated with tumor progression and metastases in a variety of gastrointestinal malignancies. VEGF is a 38 to 46-kDa dimeric N-glycoprotein that is chemotactic, as well as mitogenic, for endothelial cells in vitro, induces angiogenesis in vivo, and increases the permeability of the vascular endothelium. In humans, four different isoforms have been identified ($VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$). In pancreatic cancer, the predominant species are $VEGF_{121}$ and $VEGF_{165}$.[86]

PG-ECGF is a 55-kDa polypeptide that exists in vivo as a homodimer. PD-ECGF stimulates the chemotaxis of endothelial cells and, therefore, indirectly induces angiogenesis.[88,90] VEGF and PD-ECGF are frequently coexpressed in human cancers. Intratumoral microvessel density (MVD) is a potent prognostic marker in several tumor types, including breast cancer. Two studies in pancreatic cancer (a relatively hypovascular tumor) did not show prognostic value for MVD; the expression of PD-ECGF, however, was associated with a significantly reduced survival.[86,91] These two studies also showed no prognostic value for VEGF-expressing tumours, but a third study found significantly shorter survival for those patients with VEGF-positive tumors.[92]

Stromal factors and adhesion molecules

Urokinase plasminogen activator (uPA) and its receptor (uPAR)

Plasminogen is an inactive proenzyme that can be converted to plasmin urinary or tissue plasminogen activator (uPA and tPA). uPA appears to play a pivotal role in pericellular proteolysis during cell migration and tissue remodelling. This enzyme is released initially from cells as an inactive proenzyme (pro-uPA) that can be cleaved by serine, cysteine, and other types of proteases.[93] Pro-uPA and uPA bind to a specific cell surface receptor; following ligand binding, the uPA receptor increases the enzymatic activity of uPA itself. Plasminogen is converted to plasmin by uPA, leading to the degradation of fibrin, type IV collagen, fibronectin, and laminin. Plasmin also activates latent collagenases, such as procollagenase (matrix metalloproteinase 1 [MMP1]) and prostromelysin (MMP3).[94,95] The activation of several growth factors, such as hepatocyte growth factor, TGFβ, and bFGF, is mediated by uPA, uPAR, and plasmin. The resultant cellular activation and extracellular matrix proteolysis enhance the ability of pancreatic cancer cells to invade and metastasize. As in other tumors, there is concomitant overexpression of uPA and uPAR in pancreatic cancer.[87] Coexpression of uPA and uPAR in pancreatic cancer was associated with significantly worse survival times compared with survival times in those patients with no tumor expression of either uPA or uPAR, or neither of these molecules.[87]

E-cadherin and matrix-metalloproteinases (MMPs)

E-cadherin is a transmembrane glycoprotein that is responsible for homotypic binding and morphogenesis of epithelial tissues; it is localized to the epithelial junction complex.[96] In cancer, decreased or absent expression of E-cadherin is associated with a decrease in cellular and tissue differentiation and higher metastatic potential. Transfection of E-cadherin has been shown to inhibit the motility and invasiveness of cancer cells.

The MMPs are a family of zinc-containing proteolytic enzymes that break down extracellular matrix proteins. One of the first steps of cancer invasion is the breakdown of the basement membrane, which is composed of predominantly type IV collagen. The level of MMP enzyme activity has been shown to correspond to tumor grade, regional lymph node metastases, and distant metastases. MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are type IV collagenases and are overexpressed in pancreatic cancer.[97–99] The expression of these MMPs has been shown to directly correlate with invasion and metastasis in pancreatic cancer. A recent study assessed the expression of E-cadherin and MMP-2 and MMP-9, using in-situ hybridization, in pancreatic cancer.[88] The expression of E-cadherin in pancreatic cancer was inversely correlated with tumor progression and the development of metastases. Patients with cancers that had an expression ratio of MMP:E-cad of less than 3 had a significantly better prognosis than those with a ratio of more than 3.

Conclusion

Increasing numbers of cellular and genetic markers are under investigation in pancreatic cancer. Certain growth factors and their receptors, tumor suppressor genes, angiogenic factors, and apoptotic genes have significant correlations with survival and/or treatment responsiveness in pancreatic cancer. Although the exact role of these markers in patient management remains to be established, their investigation places pancreatic tumor biology and tumor responsiveness to present and future therapies on a fundamental and mechanistic foundation. There should be cautious optimism for newer successful approaches for pancreatic cancer treatment over the next few years.

References

1. Bramhall SR, Allum WH, Jones AG, Allwood A, Cummins C, Neoptolemos JP (1995) The incidence and treatment and survival in 13 560 patients with pancreatic cancer: an epidemiological study in the West Midlands. Br J Surg 82:111–115
2. Neoptolemos JP, Russell RCG, Bramhall S, Theis B (1997) Low mortality following resection for pancreatic and periampullary tumours in 1026 patients: UK survey of specialist pancreatic units. Br J Surg 84:1370–1376
3. Wade TP, Halaby IA, Stapleton DR, Virgo KS, Johnson FE (1996) Population based analysis of treatment of pancreatic cancer and Whipple resection: Department of Defense hospitals 1989–1994. Surgery 120:680–687
4. Neoptolemos JP, Baker P, Beger H, Link K, Pederzoli P, Bassi C, Dervenis C, Friess H, Büchler M (1997) Progress report: A randomised multicentre European study comparing adjuvant radiotherapy, 6 months chemotherapy and combination therapy versus no adjuvant treatment in resectable pancreatic cancer. Int J Pancreatol 21:97–104
5. Neoptolemos JP (1998) Adjuvant radiotherapy and follow-on chemotherapy in patients with pancreatic cancer. Results of the UK Pancreatic Cancer Study Group (UKPACA-1). GI Cancer 2:235–245
6. Geer RJ, Brennan MF (1993) Prognostic indicators for survival after resection of pancreatic adenocarcinoma. Am J Surg 165:68–72
7. Allema JH, Reinders ME, Vangulik TM, Koelemay MJW, Vanleeuwen DJ, Dewit LT, Gouma DJ, Obertop H (1995) Prognostic factors for survival after pancreaticduodenectomy for patients with carcinoma of the pancreatic head region. Cancer 75:2069–2076
8. Wade TP, Kraybill WG, Virgo KS, Johnson FE (1995) Pancreatic cancer treatment in the United States veteran from 1987–1991 — effect of tumour stage on survival. J Surg Oncol 58:104–111
9. Fortner JG, Klimstra DS, Senie RT, Maclean BJ (1996) Tumor size is the primary prognosticator for pancreatic cancer after regional pancreatectomy. Ann Surg 223:147–153
10. Sobin LH, Wittekind Ch (eds) (1997) TNM Classification of malignant tumours. Wiley-Liss, New York
11. Japan Pancreas Society (1996) Classification of pancreatic carcinoma. Kanehara, Tokyo
12. Vogel I, Kruger U, Marxsen J, Soeth E, Kalthoff H, HenneBruns D, Kremer B, Juhl H (1999) Disseminated tumor cells in pancreatic cancer patients detected by immunocytology: A new prognostic factor. Clin Cancer Res 5:593–599
13. Lundin J, Roberts PJ, Kuusela P, Haglund C (1995) Prognostic significance of serum CA 242 in pancreatic cancer. A comparison with CA19.9. Anticancer Res 15:2181–2186
14. Safi F, Schossler W, Falkenreck S, Beger H (1996) CA 19.9 serum course and prognosis of pancreatic cancer. Int J Pancreatol 20:155–161
15. Maclean GD, Reddish MA, Longnecker BM (1997) Prognostic significance of preimmunotherapy serum CA27.29 (MUC 1) mucin level after specific immunotherapy of metastatic adenocarcinoma patients. J Immunotherapy 20:70–78
16. Hahn SA, Seymour AB, Hoque ATMS, Moskaluk CA, daCosta LT, Schute M, Rozenblum E, Weinstein CL, Yeo CJ, Hruban RH, Kern SE (1995) Homozygous deletion map at 18q21.1 in pancreatic cancer. Cancer Res 55:4670–4675
17. Carraway KL, Cantley LC (1994) A neu acquaintance for erbB3 and erbB4: a role for receptor heterodimerisation in growth signalling. Cell 78:5–8
18. Prigent SA, Lemoine NR (1992) The type 1 (EGFR related) family of growth factor receptors and their ligands. Prog Growth Factor Res 4:1–24
19. Ullrich A, Schlessinger J (1990) Signal transduction by receptors with tyrosine kinase activity. Cell 203–212
20. Barton CM, Hall PA, Hughes CM, Gullick WJ, Lemoine NR (1991) Transforming growth factor alpha and epidermal growth factor in human pancreatic cancer. Br J Cancer 4:1076–1082
21. Korc M, Chandrasekar B, Yamanaka Y, Friess H, Büchler M, Beger H (1992) Overexpression of the epidermal growth factor receptor receptor in human pancreatic cancer is associated with concomitant increase in the levels of epidermal growth factor and transforming growth factor alpha. J Clin Invest 90:1352–1360
22. Yamanaka Y, Friess H, Kobrin MS, Büchler M, Beger H, Korc M (1993) Coexpression of epidermal growth factor receptor and ligands in human pancreatic cancer is associated with enhanced tumor aggressiveness. Anticancer Res 13:565–570
23. Uegaki K, Nio Y, Inoue Y, Minari Y, Sato Y, Song MM, Dong M, Tamura K (1997) Clinicopathological significance of epidermal growth factor and its receptor in human pancreatic cancer. Anticancer Res 17:3841–3847
24. Yokoyama M, Ebert M, Funatomi H, Friess H, Büchler M, Johnson GR, Korc M (1995) Amphiregulin is a potent mitogen in human pancreatic cancer cells — correlation with patient survival. Int J Oncol 6:625–631
25. Akiyama T, Sudo C, Ogawara H, Toyoshima K, Yamamoto T (1986) The product of the human c-erbB-2 gene: a 185-kilodalton glycoprotein with tyrosine kinase activity. Science 232:1644–1646
26. Mellon JK, Lunec J, Wright C, Horne CH, Kelly P, Neal DE (1996) C-erb-B2 in bladder cancer: molecular biology, correlation with epidermal growth factor receptors and prognostic value. J Urol 155:321–326
27. Hardwick Rh, Barham CP, Ozua P, Newcomb PV, Savage P, Powell R, Rahamin J, Alderson D (1997) Immunohistochemical detection of p53 and c-erbB-2 in oesophageal carcinoma; no correlation with prognosis. Eur J Oncol 23:30–35
28. Hall PA, Hughes CM, Staddon SL, Richman Pi, Gullick WJ, Lemoine NR (1990) The c-erbB-2 protooncogene in human pancreatic cancer. J Pathol 161:1995–2000
29. Lei SZ, Appert H, Nakata B, Domenico D, Kim K, Howard J (1995) Overexpression of her2/neu oncogene in pancreatic cancer correlates with shortened survival. Int J Pancreatol 17:15–21
30. Okada N, Ohshio G, Yamaki K, Imamura T, Imamura M (1995) Elevated serum c-erbB-2 protein levels in patients with pancreatic cancer: correlation to metastasis and shorter survival. Oncology 52:392–396
31. Kawesha A, Ghaneh P, Andrén-Sandberg Å, Ograed D, Skar R, Dawiskiba S, Evans JD, Campbell F, Lemoine N, Neoptolemos JP (2000) K-ras oncogene subtype mutations are associated with survival but not expression of p53, $p16^{INK4A}$, $p21^{WAF-1}$, cyclin D1, erbB-2 and erbB-3 in resected pancreatic ductal adenocarcinoma. Int J Cancer 89:469–474
32. Rajkumar T, Calvin SR, Goden, Lemoine N, Gullick W (1993) Expression of the C-erbB-3 protein in gastrointestinal tract tumours determined by monoclonal antibody RTJ1. J Pathol 170:271–278
33. Lemoine N, Lobresco M, Leung H, Barton C, Hughes C, Prigent S, Gullick W, Kloppel G (1992) The c-erbB-3 gene in human pancreatic cancer. J Pathol 168:269–273
34. Friess H, Yamanaka Y, Kobrin MS, Do DA, Büchler M, Korc M (1995) Enhanced erbB-3 expression in human pancreatic cancer correlates with tumour progression. Clin Cancer Res 1:1413–1420
35. Plowman GD, Culouscou J-M, Whitney GS, Green JM, Carlton GW, Foy L, Naubauer MG, Shoyab M (1993) Ligand specific activation of $HER4/p180^{erbB4}$, a fourth member of the epidermal growth factor family. Proc Natl Acad Sci USA 90:1746–1750
36. Tzahar E, Levkowitz G, Karunagaran D, Yi L, Peles E, Lavi S, Chang D, Liu N, Yayon A, Wen D, Yarden Y (1994) ErbB-3 and ErbB-4 function as the respective low and high affinity receptors of all Neu Differentiation Factor/Heregulin isoforms. J Biol Chem 269:25226–25233
37. Riese DJ, Bermingham Y, Van Raaij TM, Buckley S, Plowman GD, Stern DF (1996) Betacellulin activates the epidermal growth

factor receptor and erbB-4 and induces cellular response patterns distinct from those stimulated by epidermal growth factor or neuregulin-beta. Oncogene 12:343–353
38. Graber HU, Friess H, Kaufmann B, Willi D, Zimmerman A, Korc M, Buchler MW (1999) c-erbB-4 mRNA expression is decreased in non-metastatic pancreatic cancer. Int J Cancer 84:24–27
39. Bladydes JP, Schlumberger M, Wynford-Thomas D, Wyllie FS (1995) Interaction between p53 and TGF-β-1 in control of epithelial cell proliferation. Oncogene 10:307–317
40. Datto MB, Li Y, Panus JF, Howe DJ, Xiong Y, Wang XF (1995) Transforming growth factor β induces the cyclin dependent kinase inhibitor p21 through a p53 independent mechanism. Proc Natl Acad Sci USA 92:5545–5549
41. Friess H, Yamanaka Y, Büchler M, Ebert M, Beger HG, Gold LI, Korc M (1993) Enhanced expression of transforming growth factor beta isoforms in pancreatic cancer correlates with decreased survival. Gastroenterology 105:1846–1856
42. Lu Z, Friess H, Graber H, Guo X, Schilling M, Zimmermann A, Korc M, Buchler MW (1997) Presence of two signalling TGF-beta receptors in human pancreatic cancer correlates with advanced tumour stage. Dig Dis Sci 42:2054–2063
43. Coppola D, Lu L, Fruehauf J, Kyshtoobayeva A, Karl R, Nicosia S, Yeatman TJ (1998) Analysis of p53, $p21^{WAF1}$, and TGF-β1 in human ductal adenocarcinoma of the pancreas. Am J Clin Pathol 110:16–23
44. Friess H, Yamanaka Y, Büchler M, Berger HG, Kobrin MS, Baldwin RL, Korc M (1993) 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 53:2704–2707
45. Yamanaka Y, Friess H, Büchler M, Beger HG, Uchida E, Onda M, Kobrin MS, Korc M (1993) Overexpression of acidic and basic fibroblast growth factors in human pancreatic cancer correlates with advanced tumour stage. Cancer Res 53:5289–5296
46. Vickers SM, MacMillan LA, Green M, Ellis C, Thompson JA (1999) Association of increased immunostaining for inducible nitric oxide synthase and nitrotyrosine with fibroblast growth factor transformation in pancreatic cancer. Arch Surg 134:245–251
47. Ohta T, Yamamoto M, Numata M, Iseki S, Tsukioka Y, Miyashita T, Kayahara M, Nagakawa T, Miyazaki I, Nishikawa K, Yoshitake Y (1995) Expression of fibroblast growth factor and its receptor in human pancreatic carcinomas. Br J Cancer 72:824–831
48. Dong M, Nio Y, Guo KJ, Tamura K, Tian LYL, Dong YT (1998) Epidermal growth factor and its receptor as prognostic indicators in Chinese patients with pancreatic cancer. Anticancer Res 18: 4613–4619
49. Gansauge F, Gansauge S, Schmidt E, Müller J, Beger H (1998) Prognostic significance of molecular alterations in human pancreatic carcinoma — an immunohistological study. Langenbeck's Arch Surg 383:152–155
50. Kastan MB, Onykivere O, Sidransky D, Volgestein B, Craig RW (1991) Participation of the p53 protein in the cellular response to DNA damage. Cancer Res 51:6301–6311
51. Levine AJ, Momand J, Finley CA (1991) The *p53* tumour suppressor gene. Nature 351:453–456
52. Lane DP, Hollenstein M, Sidraansky D, Volgestein B, Harris CC (1991) P53 mutations in human cancers. Science 253:49–53
53. Barton CM, Staddon SL, Hughes CM, Hall PA, Sullivan C, Kloppel G, Theis B, Russel RCG, Neoptolemos J, Williamson RCN, Lane DP, Lemoine NR (1991) Abnormalities of the p53 tumour suppressor gene in human pancreatic cancer. Br J Cancer 64:1076–1082
54. Boschman CR, Stryker S, Reddy JK, Rao MS (1994) Expression of p53 in precursor lesions and adenocarcinoma of the human pancreas. Am J Pathol 145:1291–1295
55. Zhang SY, Ruggeri BA, Agarwal P, Sorling AF, Obara T, Ura H, Namiki M, Klein-Szanto AJ (1994) Immunohistochemical analysis of p53 expression in human pancreatic carcinomas. Arch Pathol Lab Med 118:150–154
56. Lundin J, Nordling S, von Boguslawsky K, Roberts PJ, Haglund C (1996) Prognostic value of immunohistochemical expression of p53 in patients with pancreatic cancer. Oncology 53:104–111
57. Nio Y, Dong M, Uegaki K, Hirahara N, Minari Y, Sasaki S, Takamura M, Iguchi C, Tamura K (1998) P53 expression affects the efficacy of adjuvant chemotherapy after resection of invasive ductal carcinoma of the pancreas. Anticancer Res 18:3773–3779
58. Nio Y, Dong M, Uegaki K, Hirahara N, Minari Y, Sasaki S, Takamura M, Iguchi C, Tamura K (1999) Comparative significance of p53 and WAF/1-p21 expression on the efficacy of adjuvant chemotherapy for resectable invasive ductal carcinoma of the pancreas. Pancreas 18:117–126
59. Serrano M, Hannon GJ, Beach D (1993) A new regulatory motif in cell cycle control causing specific inhibition of cyclin D/cdk4. Nature 366:704–707
60. Ezhevsky S, Nagahara H, Vocero-Akbani A, Gius D, Wei M, Dowdy S (1997) Hypo-phosphorylation of the retinoblastoma protein (pRb) by cyclin D:cdk4/6 complexes results in active pRb. Proc Natl Acad Sci USA 94:10699–10704
61. Medema R, Herrera R, Lam F, Weinberg R (1995) Growth suppression by $p16^{INK4}$ requires functional retinoblastoma protein. Proc Natl Acad Sci USA 92:6289–6293
62. Caldas C, Hahn SA, da Costa LT, Redston MS, Schutte M, Seymour AB, Weinstein CL, Hruban RH, Yeo CJ, Kern SE (1994) Frequent somatic mutations and homozygous deletions of the MTS1 gene in pancreatic adenocarcinoma. Nature Genet 8:27–32
63. Naumann M, Savitskaia N, Eilert C, Schramm A, Kalthoff H, Schmiegel W (1996) Frequent codeletion of p16/MTS1 and p15/MTS2 and genetic alterations in p16/MTS1 in pancreatic tumours. Gastroenterology 110:1215–1224
64. Naka T, Kobayashi M, Ashida K, Toyota N, Kaneko T, Kaibara N (1998) Aberrant p16 (INK4) expression related to clinical stage and prognosis in patients with pancreatic cancer. Int J Oncol 12:1111–1116
65. Xiong Y, Hannon GJ, Zhang H, Casso D, Kobayashi R, Beach D (1993) p21 is a universal inhibitor of cyclin kinases. Nature 366:701–704
66. Agarwal ML, Agarwal A, Taylor WR, Stark GR (1995) p53 controls both the G(2)M and the G1 cell cycle checkpoints and mediates reversible growth arrest in human fibroblasts. Proc Natl Acad Sci USA 92:8493–8497
67. Ruggeri BA, Huang L, Berger D, Chang H, Klein-Szanto AJP, Goodrow T, Wood M, Obara T, Heath CW, Lynch H (1997) Molecular pathology of primary and metastatic ductal pancreatic lesions. Analyses of mutations and expression of the p53, mdm-2, and p21/WAF1–1 genes in sporadic and familial lesions. Cancer 79:700–716
68. Song MM, Nio Y, Sato Y, Tamura K, Furuse K (1996) Clinicopathological significance of Ki-ras point mutation and p21 expression in benign and malignant exocrine tumours of the human pancreas. Int J Pancreatol 20:85–93
69. Smit VTHBM, Boot AJM, Smits AMM, Fleuren GJ, Cornelisse CJ, Bos JL (1988) K-ras codon 12 mutations occur very frequently in pancreatic adenocarcinomas. Nucleic Acids Res 16:7773–7782
70. Lemoine NR, Jain S, Hughes CM, Staddon SL, Maillet B, Hall PA, Kloppel G (1992) Ki-ras oncogene activation in preinvasive pancreatic cancer. Gastroenterology 102:230–236
71. Finkelstein SD, Przygodzki R, Pricolo VE, Sayegh R, Bakker A, Swalsky PA, Keller G (1994) K-*ras* topographic genotyping of pancreatic adenocarcinoma. Arch Surg 129:367–373
72. Hruban RH, van Mansfield AD, Offerhaus GJ, van Weering DH, Allison DC, Goodman SN, Kensler TW, Bose KK, Cameron JL, Bos JL (1993) K-*ras* oncogene activation in adenocarcinoma of the human pancreas. A study of 82 carcinomas using a combination of mutant enriched polymerase chain reaction analysis and allele specific oligonucleotide hybridisation. Am J Pathol 143: 545–554

73. Dergham ST, Dugan MC, Kucway R, Du W, Kamarauskiene DS, Vaitkevicius VK, Crissman JD, Sarkar FH (1997) Prevalence and clinical significance of combined K-ras mutation and p53 aberration in pancreatic adenocarcinoma. Int J Pancreatol 21: 127–143
74. Kato J-Y, Matsushime H, Hiebert SW, Ewen ME, Sherr CJ (1993) Direct binding of cyclin D to the retinoblastoma gene product pRb and pRb phosphorylation by the cyclin dependent kinase cdk4. Genes Dev 7:331–342
75. Scherr CJ, Roberts JM (1995) Inhibitors of mammalian G1 cyclin dependent kinases. Genes Dev 9:1149–1163
76. Hall M, Peters G (1996) Genetic alterations of cyclins, cyclin-dependent kinases and cdk inhibitors in human cancer. Adv Cancer Res 68:67–108
77. Wolfel TM, Schneider J, Serrano M, Wolfel C, Klehmann-Hieb E, De Plaen E, Hankeln T, Meyerzum Buschenfelde KH, Beach D (1995) A p16INK4A-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. Science 269:1281–1284
78. Reed JC (1998) Bcl-2 and other inhibitors of apoptosis: mechanisms of action. Br J Cancer 78:S16 Suppl 1
79. Boise LH, Gonzalez-Garcia M, Postema CE, Ding L, Lindstein T, Turka LA, Mao X, Nunez G, Thompson CB (1993) Bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell 74:597–608
80. Korsmeyer SJ (1996) Apoptosis; regulation and oncogenesis. Proceedings of the American Academy of Cancer Researchers. 37:624–625
81. Friess H, Lu Z, Graber H, Zimmermann A, Adler G, Korc M, Schmid RM, Buchler MW (1998) Bax but not bcl-2 influences the prognosis of human pancreatic cancer. Gut 43:414–421
82. Evans JD, Cornford P, Dodson A, Foster C, Neoptolemos JP (1998) Expression of bcl-xL correlates with survival in pancreatic cancer. (abstract) Br J Surg 85:1589
83. Friess H, Lu Z, Andrén-Sandberg Å, Berberat P, Zimmerman A, Adler G, Schmid R, Buchler MW (1998) Moderate activation of the apoptosis inhibitor bcl-xL worsens the prognosis in pancreatic cancer. Ann Surg 228:780–787
84. Miyamoto Y, Hosotani R, Wada M, Lee J, Koshiba T, Fujimoto K, Tsuji S, Nakajima S, Doi R, Kato M, Shimada Y, Imamura M (1999) Immunohistochemical analysis of bcl-2, bax, bcl-x and mcl-1 expression in pancreatic cancers. Oncology 56:73–82
85. Shimoyama S, Gansauge F, Gansauge S, Negri G, Oohara T, Beger H (1996) Increased angiogenin expression in pancreatic cancer is related to cancer aggressiveness. Cancer Res 56:2703–2706
86. Fujimoto K, Hosotani R, Wada M, Lee JU, Koshiba T, Miyamoto Y, Tsuji S, Nakajima S, Doi R, Imamura M (1998) Expression of two angiogenic factors, vascular endothelial growth factor and platelet derived endothelial cell growth factor in human pancreatic cancer and its relationship to angiogenesis. Eur J Cancer 34: 1439–1447
87. Cantero D, Friess H, Deflorin J, Zimmermann A, Bründler M-A, Riesle E, Korc M, Buchler MW (1997) Enhanced expression of urokinase plasminogen activator and its receptor in pancreatic carcinoma. Br J Cancer 75:388–395
88. Kuniyasu H, Ellis LM, Evans DB, Abbruzzese JL, Fenoglio CJ, Bucana CD, Cleary KR, Tahara E, Fidler IJ (1999) Relative expression of E-cadherin and type IV collagenase genes predicts disease outcome in patients with resectable pancreatic carcinoma. Clin Cancer Res 5:25–33
89. Hu G-F, Riordan JF, Vallee BL (1994) Angiogenin promotes invasiveness of cultured endothelial cells by stimulation of cell-associated proteolytic activities. Proc Natl Acad Sci USA 91: 12096–12100
90. Ishikawa F, Miyazono K, Hellman U, Drexler H, Wernstedt C, Hagiwara K (1989) Indentification of angiogenic activity and the cloning and expression of platelet-derived endothelial cell growth factor. Nature 338:557–562
91. Ellis LM, Takahashi Y, Fenoglio C, Cleary K, Bucana C, Evans DB (1998) Vessel counts and vascular endothelial growth factor expression in pancreatic adenocarcinoma. Eur J Cancer 34:337–340
92. Ikeda N, Adachi M, Taki T, Huang C, Hashida H, Takabayashi A, Sho M, Nakajima Y, Kanehiro H, Hisanaga M, Nakano H, Miyake M (1999) Prognostic significance of angiogenesis in pancreatic cancer. Br J Cancer 79:1553–1563
93. Schmitt M, Janicke F, Graeff H (1992) Tumour associated proteases. Fibrinolysis 6:3–26
94. Lim YT, Sugiura Y, Laug WE, Sun B, Garcia A, DeClerk YA (1996) Independent regulation of matrix metalloproteinase and plasminogen activators in human fibrosarcoma cells. J Cell Phys 167:333–340
95. Baramova EN, Bajou K, Remacle A, L'Hoir C, Krell HW, Weidle UH, Noel A, Foidart JM (1997) Involvement of PA/plasmin system in the processing of pro-MMP9 and in the second step of pro-MMP2 activation. FEBS Lett 405:157–162
96. Takeichi M (1991) Cadherin cell adhesion receptor as a morphogenic regulator. Science 251:1451–1455
97. Bramhall SR, Stamp GWH, Dunn J, Lemoine NR, Neoptolemos JP (1996) Expression of collagenase (MMP2), stromelysin (MMP3) and tissue inhibitor of the metalloproteinases (TIMP1) in pancreatic and ampullary disease. Br J Cancer 73:972–978
98. Bramhall SR, Neoptolemos JP, Stamp GWH, Lemoine NR (1997) Imbalance of expression of matrix metalloproteinases (MMPs) and tissue inhibitors of the matrix metalloproteinases (TIMPs) in human pancreatic carcinoma. J Pathol 182:347–355
99. Koshiba T, Hostani R, Wada M, Miyamoto Y, Fujimoto K, Lee JU, Doi R, Arii S, Imamura M (1998) Involvement of MMP2 activity in invasion and metastases of pancreatic cancer. Cancer 82:642–650