ELSEVIER

European Journal of Cancer 41 (2005) 2213–2236

European Journal of Cancer

www.ejconline.com

Review

Molecular prognostic markers in pancreatic cancer: A systematic review

G. Garcea [a,*], C.P. Neal [a], C.J. Pattenden [b], W.P. Steward [a], D.P. Berry [b]

[a] *Cancer Studies and Molecular Medicine, The Robert Kilpatrick Clinical Sciences Building, University of Leicester, The Leicester Royal Infirmary, Leicester LE2 7LX, UK*

[b] *Department of Hepatobiliary and Pancreatic Surgery, The Leicester General Hospital, Gwendolen Road, Leicester LE5 4PW, UK*

Received 7 April 2005; accepted 8 April 2005
Available online 16 September 2005

Abstract

Pancreatic cancer is one of the most lethal tumours of the gastrointestinal tract. The ability to predict which patients would benefit most from surgical intervention and/or chemotherapy would be a great clinical asset. Considerable research has focused on identifying molecular events in pancreatic carcinogenesis, and their correlation with clinicopathological variables of pancreatic tumours and survival. This systematic review examined evidence from published manuscripts looking at molecular markers in pancreatic cancer and their correlation with tumour stage and grade, response to chemotherapy and long-term survival. A literature search was undertaken using PubMed and MEDLINE search engines, using the keywords p53, p21, p16, p27, SMAD4, K-ras, cyclin D1, Bax, Bcl-2, EGFR, EGF, c-erbB2, HB-EGF, TGFβ, FGF, MMP, uPA, cathepsin, heparanase, E-cadherin, laminins, integrins, TMSF, CD44, cytokines, angiogenesis, VEGF, IL-8, β-catenin, DNA microarray, and gene profiling. A bewildering number of biomarkers are currently under evaluation. For the most part, the evidence regarding their application as prognostic indicators is conflicting. The advent of gene microarray and mass spectrometric protein profiling offers the potential to examine many different biomarkers simultaneously. This 'protein/gene signature' could revolutionise work in this field and allow researchers to develop accurate and reproducible predictions of survival based on protein or gene profiles.

Keywords: Pancreatic cancer; Prognosis; Survival; Biomarkers

1. Introduction

Pancreatic cancer is notorious for its late presentation, early and aggressive local invasion, metastatic potential and poor outcome [1–4]. Histologically, the pancreas is divided into the exocrine pancreas, consisting of ducts and acini, and the endocrine pancreas, consisting of hormone-secreting cells, arranged in islets. Most pancreatic cancer arises from the exocrine pancreas. Tumours originating from the epithelium lining the pancreatic duct represent 85% of pancreatic cancers [5] and form the focus of this review. Only 20% of pancreatic cancers are amenable to surgical resection at presentation [3,6] and despite the medical advances made over the last 20 years, pancreatic cancer would appear to have benefited the least in terms of survival.

Current staging systems used to predict survival from pancreatic cancer suffer from geographical variations in how staging protocols are applied, making comparison of survival data from global studies difficult. Tumour markers have also been evaluated, but their application in early disease and operable tumours is questionable, since they rely on a significant tumour burden to be present before reliable quantifiable levels are achieved.

* Corresponding author.
E-mail address: gg43@le.ac.uk (G. Garcea).

0959-8049/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2005.04.044

Predicting prognosis for patients with pancreatic cancers may identify those who could benefit from aggressive intervention including surgery and/or chemotherapy. Molecular markers have the advantage of being quantifiable signatures along the pancreatic carcinogenesis cascade, and hence measurement of these markers may be standardised. In addition, markers that display prognostic significance offer the potential to become targets for intervention in themselves, and so offer novel therapeutic strategies in the management of pancreatic cancer. Molecular markers could also be measured in surrogate tissue such as sera, allowing monitoring of patient progression following tumour resection or their response to medical therapy. Finally, these molecular markers could be applied to achieving an earlier diagnosis of pancreatic tumours, or in identifying patients at-risk of pancreatic cancer development. These measures would allow close monitoring of at-risk patients, and so provide crucial information for understanding pancreatic cancer progression better.

This review examined the available evidence, from published manuscripts, on molecular alterations found in pancreatic cancer and their implications for prognosis. Studies examining the correlation of biomarkers with clinicopathological features that could also influence prognosis, i.e. response to chemotherapy, or relationship with tumour stage and grade, were also included. Only studies that examined markers in human pancreatic tissue or from surrogate tissue (for example sera) were included in the review. Animal and *in vitro* work was excluded. Endocrine pancreatic cancers were also excluded. A literature search was performed using PubMed and MEDLINE search engines, with the keywords p53, p21, p16, p27, SMAD4, K-ras, cyclin D1, Bax, Bcl-2, EGFR, EGF, c-erbB2, HB-EGF, TGFβ, FGF, MMP, uPA, cathepsin, heparanase, E-cadherin, laminins, integrins, TMSF, CD44, cytokines, angiogenesis, VEGF, IL-8, β-catenin, DNA microarray and gene profiling.

2. Oncogenes and tumour suppressor genes

Oncogenes and tumour suppressor genes control cell cycle and apoptosis. Events leading to carcinogenesis involve mutations in oncogenes, resulting in dominant gain of function, or mutations in tumour suppressor genes with a resultant loss of their inhibitory action. In pancreatic cancer pathogenesis, activation of the *K-ras* oncogene has been recorded in 80% of cases, and inactivation of the tumour suppressor genes *p16*, *p53* and *DPC4* in more than 60% of pancreatic cancers [7,8].

2.1. Tumour suppressor gene p53

p53 controls the cell cycle at the G1/S interface and plays an important role in inducing programmed cell death in response to severe damage to cellular DNA [9]. Mutations in the *p53* gene lead to accumulation of the mutant protein within the cell nucleus. Many anticancer agents exert their clinical action by inducing apoptosis in tumour cells, and hence *p53* positivity in pancreatic tumours could influence the efficacy of anticancer agents. Data on *p53* in relation to pancreatic cancer survival and sensitivity to chemotherapy are outlined in Tables 1 and 2 [10–28].

The data show that the evidence relating *p53* expression to survival is conflicting. For the most part, however, studies have failed to show a convincing correlation of *p53* mutation with decreased survival. Although Hu and colleagues [14] and Nio and colleagues [16] found that *p53* expression coupled with *Bcl-2* and *p*21, respectively, could predict survival. The reasons for these conflicting results may be that the absence of *p53* expression is not always synonymous with normal function of the *p53* gene.

The role of *p53* in the cell cycle should result, intuitively, in *p53* mutations decreasing a tumour's sensitivity of chemotherapy due to loss of the apoptotic function conferred by wild-type *p53*. However, the data in Table 1, suggest that *p53* mutations may increase chemosensitivity. It has been postulated that *p53* inactivation, through mutation, may render tumour cells more sensitive to certain anti-cancer agents, such as cisplatin, due to loss of ability to repair drug-induced DNA damage [29–31]. However, DNA synthesis blockers, such as 5-fluorouracil, induce apoptosis through *p53*-dependent mechanisms, and hence loss of *p53* function could result in decreased sensitivity to certain types of chemotherapy agents. In light of the present evidence, *p53* mutations alone are unlikely offer useful prognostic information in patients with pancreatic cancer, although they may be used to select patients more likely to respond to adjuvant chemotherapy.

2.2. Tumour suppressor gene p16

The cyclin-dependent kinases CDK4 and CDK6 normally interact with cyclin D to phosphorylate the retinoblastoma (Rb) protein. This phosphorylation of Rb allows it to dissociate from a complex formed with the protein, elongation factor 2 (E2F1), allowing E2F1 to activate genes required for DNA synthesis needed for forward progression along the cell cycle [32]. *p16* acts as an inhibitor of CDK4 and CDK6, and so plays a key role in controlling the G1 checkpoint in the cell cycle [32]. Loss of expression of *p16* is observed in most pancreatic tumours [10,25,33–35] (Table 3). For the most part, studies examining loss of *p16* expression have found an association with decreased survival, increased tumour size and increased risk of metastases. Loss of *p16* appears to be a relatively early event in the progression of pancreatic cancer [36], and this may be responsible for its ability

Table 1
Evidence for *p53* expression with survival

Study	Year	Patients (*n*)	Gene/gene product	Method	Increased or decreased expression (%)	*p53* expression alone and correlation with survival	Significance
Biankin *et al.* [25]	2002	125	p53 protein	IHC	↑ 54.0	No prognostic association	–
Dong *et al.* [18]	2000	59	p53 protein	IHC	↑ 69.5	No prognostic association	–
Campani *et al.* [26]	1999	133	p53 protein	IHC	↑ 54.0	*p53* expression in lymph nodes associated with reduced survival	$P = 0.05$
Nio *et al.* [17]	1999	58	p53 protein	IHC	↑ 50.0	Weak correlation	Not significant
Hu *et al.* [14]	1999	52	p53 protein	IHC	↑ 61.5	No prognostic association	$P < 0.05$
Naka *et al.* [10]	1998	32	p53 protein	IHC	↑ 59.0	No prognostic association	–
Makinen *et al.* [28]	1998	74	p53 protein	IHC	↑ 40.0	No prognostic association	–
Ohshio *et al.* [27]	1998	81	p53 protein	IHC	↑ 54.0	No prognostic association	–
Ruggeri *et al.* [24]	1997	136	Nuclear p53 protein	IHC	↑ 56.0	No prognostic association or correlation with tumour stage	–
Kawesha *et al.* [11]	1997	142	p53 protein	IHC	↑ 35.0	No prognostic association	–
Dergham *et al.* [19]	1997	76	p53 protein	IHC	↑ 43.0	No prognostic association	–
Lundin *et al.* [15]	1996	133	p53 protein	IHC	↑ 47.0	No prognostic association	–
Nakamori *et al.* [21]	1995	37	*p53* gene	Direct sequencing	↑ 43.0	Correlation with decreased survival	$P = 0.02$
Yokoyama *et al.* [20]	1994	69	p53 protein and gene	IHC and Western blotting	↑ 58.0	Correlation with decreased survival	$P < 0.05$
Zhang *et al.* [22]	1994	54	p53 protein	IHC	↑ 37.0	No prognostic association	–
DiGiuseppe *et al.* [23]	1994	48	p53 protein	IHC	↑ 48.0	No prognostic association	–

IHC, immunohistochemical method.

Table 2
Evidence for p53 expression and sensitivity to chemotherapy

Study	Year	Patients (*n*)	Gene/gene product	Method	Increased or decreased expression (%)	*p53* expression alone and correlation with survival	Response to chemotherapy (ACT)	Significance
Dong *et al.* [12]	2003	72	*p53* gene	Direct sequencing	↑ 62.5	No prognostic association	Better survival ratio in patients with *p53* mutation	Not significant
Nio *et al.* [13]	2001	63	p53 protein	IHC	↑ 50.8	No prognostic association	Better survival with ACT in *p53*+ group	$P = 0.024$
Nio *et al.* [16]	1998	58	p53 protein	IHC	↑ 50.0	No prognostic association	Affects efficacy of chemotherapy	–
Sinicrope *et al.* [74]	1996	35	p53 protein	IHC	↑ 55.0	No prognostic association	No prognostic association	–

IHC, immunohistochemical method; ACT, adjuvant chemotherapy.

Table 3
Data from p16 protein analysis and outcome from pancreatic cancer

Study	Year	Patients (*n*)	Gene/gene product	Method	Proportion of positivity for p16 protein	*p16* expression alone and correlation with survival	*p16* expression and tumour stage	Significance
Ohtsubo *et al.* [33]	2003	60	p16 protein and gene	IHC and PCR	Strongly + in 37%	Shorter survival in patients with *p16* mutation or hypermethylation	Tumours larger in patients with decreased expression of *p16* protein	$P < 0.05$
Gerdes *et al.* [34]	2002	62	p16 protein and gene	IHC and PCR	27% + for mutations	p16 protein mutation associated with decreased survival	–	$P < 0.05$
Biankin *et al.* [25]	2002	98	p16 protein	IHC	31% + for p16	No correlation with survival	No correlation with tumour stage	NS
Naka *et al.* [10]	1998	32	p16 protein	IHC	59% + for p16	Lack of *p16* expression correlated with decreased survival	Lack of *p16* expression correlated with increasing stage	$P < 0.05$
Kawesha *et al.* [11]	1998	142	p16 protein	IHC	13% + for p16	No correlation with survival	No correlation with stage	–
Hu *et al.* [35]	1997	20	p16 protein	IHC	37.5% + for p16	Shorter survival and increased risk of metastases in cases with no *p*16 expression	Associated with histological grade	$P < 0.05$

IHC, immunohistochemical method; PCR, polymerase chain reaction; NS, not significant.

Table 4
Data from p21 protein analysis and outcome from pancreatic cancer

Study	Year	Patients (*n*)	Gene/gene product	Method	Proportion of positivity for p21 protein (%)	*p21* expression alone and correlation with survival	*p21* expression and tumour stage	Significance
Biankin *et al.* [25]	2002	125	p21 protein	IHC	79	No correlation with survival	No correlation with tumour stage	NS
Hashimoto *et al.* [39]	2001	62	p21 protein	IHC	40	*p*21+ patients had a higher survival curve, but not statistically significant	N/A	NS
Song *et al.* [40]	2000	85	p21 protein	IHC	59 (mean)	No correlation	N/A	–
Ahrendt *et al.* [41]	2000	90	p21 protein	IHC	56	*p*21+ patients associated with longer survival following chemotherapy	N/A	$P = 0.01$
Nio *et al.* [17]	1999	58	p21 protein	IHC	41	*p21*+ patients showed a higher survival curve, but not statistically significant	N/A	NS
Coppola *et al.* [42]	1998	42	p21 protein	IHC	38	No correlation with survival	Correlated with grade, but not stage	NS
Dergham *et al.* [45]	1997	81	p21 protein	IHC	49 (mean)	*p21*+ patients with no family history of cancer, showed improved survival trends	N/A	$P = 0.029$
Dergham *et al.* [19]	1997	75	p21 protein	IHC	57	*p*21+ patients found to have a better median survival, but not significant	Associated with earlier stage	$P = 0.23$
Song *et al.* [40]	1996	44	p21 protein	IHC	43	*p21* mutations showed the worst survival following pancreatectomy	N/A	NS
Yamaguchi *et al.* [44]	1989	96	p21 protein	IHC	52	No relationship with *p21* and outcome	N/A	–

IHC, immunohistochemical method; NS, not significant.

to be a more significant prognostic indicator of survival in patients with pancreatic cancer.

2.3. Tumour suppressor gene p21

p21 is a member of the cip/kip family and functions as an inhibitor of cyclin-dependent kinases [37]. The 21 kDa protein transcripted by the WAFI gene forms complexes with cyclinA/CDK2 and cyclinD1/CDK4 and so inhibits their activity *in vitro*. *p21* also has binding sites for the proliferating cell nuclear antigen (PCNA), and so by inhibiting nuclear sequestration of the protein, may further inhibit cell growth. *p21* is a down-stream target of *p53* activation and allows time for repair to damaged DNA, via G1 arrest [38]. Loss of *p21* activity has been observed in approximately 30–60% of pancreatic tumour specimens [17,19,25,39–45] (Table 4). However, most studies thus far, have not found a convincing relationship between *p21* and survival from pancreatic cancer.

2.4. Tumour suppressor gene SMAD4/DPC4

SMAD4, also know as *DPC4*, was originally isolated from the human chromosome 18q21 [46]. Loss of expression has been observed in around 54% of pancreatic cancer specimens [46] and around 33% of patients with pancreatic dysplasia [47]. *SMAD4* is a member of the SMAD family and regulates transduction of the TGFβ superfamily [48] and angiogenesis [49]. The major biological activity of TGFβ is its potent inhibition of cell proliferation, via G1 arrest and hence loss of activity of *SMAD4* results in the loss of a major component of cell growth suppression.

Despite one report, which found that loss of *SMAD4* expression resulted in significantly shorter survival following resection, other studies have failed to corroborate this [25,50,51] (Table 5). In fact, Biankin and colleagues found the opposite to be true with *DPC4*-negative patients demonstrating longer survival time following resection [25]. One possible reason for this apparently discrepant result is that immunohistochemical analysis may not differentiate between wild-type and mutated *SMAD4* proteins. Further work is needed to elucidate further the exact relationship between *SMAD4* and prognosis.

2.5. Tumour suppressor gene p27

p27 is a cyclin-dependent kinase (CDK) inhibitor and regulates cell cycle progression from the G1 to the S phase [52]. Although loss of *p27* expression is a rare event in carcinogenesis, it has been observed for a number of tumours, including pancreatic cancers. Present data on the influence of *p27* on outcome for pancreatic cancer are limited, but there is some evidence that *p27* mutations may influence survival [52–54] (Table 5).

2.6. Tumour oncogene K-ras

ras mutations are found in 80–90% of pancreatic cancer patients, usually at codon 12 of the *K-ras* gene [55–57]. The high frequency of *K-ras* mutations has led to speculation regarding its application as a diagnostic tumour marker, as well as a prognostic indicator. The *ras* family of oncogenes encode for proteins with GTPase activity and act as 'switches' in signal transduction [58]. Most of the evidence, so far, suggests that *K-ras* mutations are not significantly associated with survival in pancreatic cancer patients (Table 6) [11,18,19,40,43,59–64]. Only two studies found that *K-ras* mutation offered prognostic information post resection [59,63]. However, the subtype [11] or number of *K-ras* mutations [43,61] may offer greater information regarding survival.

Kawesha and colleagues [11] looked at the largest number of pancreatic cancer specimens, so far recorded, and found that GaT, cGT and GcT mutations demonstrated shorter median survival times when compared with other mutations. Other studies examining *K-ras* mutations in colorectal cancer subjects have also shown that the subtype of *K-ras* mutations is linked to survival, with G → A and G → C tranversions having a shorter survival than G → T tranversions [65].

2.7. Tumour oncogene cyclin D1

Cyclin D1 regulates the G1/S transition phase of the cell cycle. There is a T-cell factor (TCF) binding site within the *cyclin D1* promoter region, and transcription is activated by the β-catenin/TCF complex [66]. Elevated levels of *cyclin D1* reduce the dependency of cells on exogenous mitogens and shorten the G1 phase. *Cyclin D1* has been linked to a number of different cancers, including colorectal, oesophageal, gastric, lung, head and neck, as well as pancreatic cancers. The data on *cyclin D1* and survival are summarised in Table 7 [11,67–71].

3. Apoptosis

3.1. Bcl-2 and Bax

Apoptosis refers to the process of programmed cell death, which acts to limit cell proliferation in normal tissue. There are many apoptotic pathways, but the best-studied are the *bcl-2* family of apoptotic genes. Bcl-x belongs to the Bcl-2 family of proteins, other members being Bcl-2, Bcl-w and Mcl-1. Bcl-x exists as two isoforms, Bcl-xL and Bcl-xS. Bcl-xL acts as an inhibitor

Table 5
Loss SMAD4/DPC4 and *p27* expression and survival from resected pancreatic cancers

Study	Year	Patients	Gene/gene product	Method	Number of patients with loss of DPC4/SMAD4 expression (%)	Correlation with survival	Correlation with tumour stage	Significance
Hua *et al.* [50]	2003	34	DPC4 protein	IHC	23.5	Loss of DPC4 expression associated with shorter survival	Loss of DPC4 expression more probable with later stages (IV)	NS
Biankin *et al.* [25]	2002	129	DPC4 protein	IHC	53	Loss of DPC4 expression associated with longer survival following resection	Loss of DPC4 expression associated with early tumour stage	$P = 0.0257$
Tascilar *et al.* [51]	2001	249	SMAD4 protein and gene	IHC and gene sequencing	55	SMAD4 preservation resulted in longer survival times; 19.2 months *versus* 14.7 months	–	$P = 0.03$
					Number of patients with loss of *p27* expression (%)	Correlation with median survival		
Juuti *et al.* [53]	2003	143	p27 protein	IHC	70.1	Five-year survival rate 3.6% in p27 negative patients compared with 20 for p27 positive	–	$P = 0.03$
Feakins *et al.* [54]	2003	46	p27 protein	IHC	–	Trend towards worse survival, but not an independent prognostic factor	No correlation with tumour stage	NS
Lu *et al.* [52]	1999	35	p27 protein	IHC	46	Loss of p27 expression associated with poorer survival	–	$P = 0.024$

IHC, immunohistochemical method.

Table 6
K-ras mutations and survival in pancreatic cancer

Study	Year	Patients	Gene/gene product	Method	Number of patients with mutation/protein	Correlation with survival	Correlation with tumour stage	Significance
Kitago *et al.* [61]	2004	20 IPMT[c] 7 cancer	Gene mutation	PCR and SSCP	80% of IPMT and 100% of ductal cancers	Survival of IPMT patients with two *K-ras* mutations better than that where patients had only one *K-ras* mutation	–	$P < 0.0021$
Dong *et al.* [18]	2000	59	Gene mutation	Dot blot hybridisation	76.3%	Not alone, but combined with p53 predicted poor prognosis	–	$P = 0.027$
Kawesha *et al.* [11]	2000	157	Gene mutation	PCR and SSCP	75%	No overall association with survival, but significant differences found between subtype of *K-ras* mutation and survival	–	$P = 0.0007$
Song *et al.* [43]	2000	85 total	Gene mutation	Dot blot hybridisation	82.5% (mean)	Differences in frequency of *K-ras* mutation found between Japanese and Chinese patients, but no association with survival	–	NS
Castells *et al.*[a] [59]	1999	44	Gene mutation	PCR and SSCP[b]	27%	Independent prognostic factor in survival for pancreatic cancer patients	Associated with tumour stage (presence of distant metastases)	$P = <0.05$
Allison *et al.* [60]	1998	86	Gene mutation	Dot blot hybridisation	–	No correlation with survival	No correlation with tumour stage	–
Dergham *et al.* [19]	1997	76	Gene mutation	Dot blot hybridisation	72%	No correlation	No correlation	$P = 0.024$
Song *et al.* [43]	1996	44 primary and 15 metastatic lesions	Gene mutation	Dot blot hybridisation	97% of primary lesions and 60% of metastatic lesions	Patients with single *K-ras* mutation had a better survival than those with double mutations following pancreatectomy.	–	$P < 0.05$
Finkelstein *et al.* [63]	1994	55 primary and 56 metastases	Gene mutation	Dot blot hybridisation	56% primary and 88% metastases	*K-ras* mutation associated with shorter survival following resection 8.2 months *versus* 21.3 months	No correlation with tumour stage	$P < 0.05$
Hruban *et al.* [64]	1993	82	Gene mutation	PCR and SSCP	83%	No correlation with survival	No correlation with tumour stage	–
Motojima *et al.* [62]	1991	53	Gene mutation	Dot blot hybridisation	87%	No correlation with survival	No correlation with tumour stage	–

PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism.
[a] *K-ras* mutations in DNA extracted from plasma of patients with pancreatic cancer.
[b] Single-strand conformation polymorphism techniques.
[c] Intraductal papillary mucinous tumours.

Table 7
Cyclin D1 and prognosis in pancreatic cancer

Study	Year	Patients	Gene/gene product	Method	Number of patients increased *cyclin D1* (%)	Correlation with survival	Correlation with tumour stage	Significance
Li *et al.* [70]	2003	47	*Cyclin D1*	IHC	74.5	No correlation	No correlation	–
Qiao *et al.* [69]	2001	43	*Cyclin D1*	IHC	65.71	No correlation	–	–
Kawesha *et al.* [11]	2000	157	*Cyclin D1*	IHC	72	No correlation	No correlation	–
Gansauge *et al.* [68]	1998	82	*Cyclin D1*	IHC	69	Shorter survival in *cyclin D1* positive patients	–	$P < 0.05$
Kornmann *et al.* [71]	1998	32	*Cyclin D1* mRNA	Reverse PCR	67	Median survival of 15.5 months versus 6.5 months for patients with higher *cyclin D1* expression	–	$P < 0.007$
Gansauge *et al.* [67]	1997	67	Gene and gene product	IHC and PCR	82% mRNA by PCR and 68.4% by IHC	Significant association with poorer prognosis	–	$P < 0.01$

PCR, polymerase chain reaction; IHC, immunohistochemical method.

to apoptosis, whilst Bcl-xS acts as a promoter. Pro-apoptotic proteins, such as Bax, dimerise with the Bcl proteins and the ratio of the Bcl proteins with the pro-apoptotic Bax subsequently determine cell death or survival [72,73].

Apoptosis-related proteins may affect outcome in pancreatic carcinoma directly, or by influencing the susceptibility of tumour cells to chemotherapeutic regimens. Nearly all chemotherapeutic agents rely on endogenous apoptotic mechanisms to induce cell death, and so the expression of these gene products could have important prognostic implications in determining responsiveness to chemotherapy agents.

There is strong clinical data supporting a positive correlation between Bcl-2 expression (an anti-apoptotic gene) and survival following pancreatic cancer resection (Table 8) [13,14,27,28,74–80], although some studies have found no correlation, or a negative relationship between the two [75,77–79]. Data regarding Bax expression and survival is as yet insufficient to make any firm conclusions. The observation that Bcl-2 positivity, an anti-apoptotic factor, results in longer survival is surprising and may only be explained when more information regarding the role of other members of the Bcl-2 family is obtained.

3.2. Survivin

Survivin is a recently described member of the family of inhibitors of apoptosis proteins (IAP). Survivin is found only in tumour tissue, and is undetectable in normal cells. Survivin has been found in 60–80% of pancreatic tumour specimens [81–84] and has also been implicated in radioresistance of pancreatic cancer cell lines [85]. Survivin expression has been shown to correlate with histological grade and clinical stage of pancreatic cancer [84], proliferation index [83] and in one study survivin has been shown to be an independent prognostic indicator of overall survival [81].

4. Growth factors and growth factor receptors

Growth factors are a group of molecules that transmit signals between cells and function as stimulators or inhibitors of cell division, differentiation and migration. Many different families of growth factors exist, but the most studied in relation to pancreatic cancer are discussed below. Growth factors relating specifically to angiogenesis will be discussed later.

4.1. Transforming growth factor beta (TGFβ) and receptors

TGFβ refers to a superfamily of polypeptide growth factors that influence a number of processes in both

Table 8
The Bcl-2 family and Bax genes/proteins and association with survival and response to chemotherapy in pancreatic cancer

Study	Year	Patients	Gene/gene product	Method	Number of patients with mutation/protein	Correlation with survival	Correlation with tumour stage	Correlation with efficacy of chemotherapy
Sun *et al.* [75]	2002	97	Bcl-2 protein	IHC	72% positive	Lower survival in Bcl-2 positive	Bcl-2 positive staining lower in higher stage or metastatic disease (significant)	–
Nio *et al.* [76]	2001	66	Bax protein	IHC	64%	Bax positive patients exhibited better survival that Bax negative	Negative correlation with nodal involvement (significant)	–
			Bcl-2 protein		24%	Bcl-2 positive patients showed better survival that Bcl-2 negative	No correlation	–
Nio *et al.* [13]	2001	63	Bcl-2 protein	IHC	25.4%	Bcl-2 positive patients showed a better survival than Bcl-2 negative patients	–	No correlation
Evans *et al.* [77]	2001	23	Bcl-2 protein Bax protein Bcl-x protein		Not detected –	Survival determined by relative level of Bcl-X expression. Strong expression associated with 171 d median survival compared with 912 d in patients with reduced expression	–	–
Campani *et al.* [78]	2001	120 primary cancers and 43 lymph node metastases	Bcl-2 protein	IHC	25% of primary tumours positive and 7% of lymph node metastases	No correlation	Well-differentiated tumours more frequently Bcl-2 positive ($P = 0.002$)	–
Hu *et al.* [14]	1999	52	Bcl-2 protein	IHC	23.1%	Bcl-2 negative staining correlated with increased survival[a]	Bcl-2 negative staining associated with increasing histological grade and clinical stage	–
Friess *et al.* [79]	1998	60	Bcl-2 protein and mRNA	IHC and Northern Blot	30% by Northern blotting and 28% by IHC	No correlation	–	–
			Bax protein and mRNA	IHC and Northern Blot	61% by Northern Blot and 83% by IHC	Strong predictor of survival ($P < 0.039$)	–	–
Friess *et al.* [80]	1998	74	Bcl-xL protein and mRNA	IHC and Northern Blot	54% by Northern Blot and 88% by IHC	Weak Bcl-xL expression correlated with significantly longer survival after resection than strong Bcl-xL expression; 12 *versus* 5 months ($P < 0.05$)	–	–

(*continued on next page*)

Table 8 (*continued*)

Study	Year	Patients	Gene/gene product	Method	Number of patients with mutation/protein	Correlation with survival	Correlation with tumour stage	Correlation with efficacy of chemotherapy
Ohshio *et al.* [27]	1998	81	Bcl-2 protein	IHC	55%	No correlation with survival	Bcl-2 positivity associated in higher grade pancreatic tumours	–
Makinen *et al.* [28]	1998	74	Bcl-2 protein	IHC	52%	Bcl-2 expression associated with longer survival ($P = 0.08$)	No correlation with stage or grade	–
Sinicrope *et al.* [74]	1996	40	Bcl-2 protein	IHC	55%	Better survival trend in bcl-2 positive patients ($P = 0.06$)	No correlation	No correlation

IHC, immunohistochemical method.
[a] Subgroup of *p53* positive and Bcl-2 negative.

normal and tumourigenic cells, including regulation of cell growth, cell differentiation, angiogenesis, cell invasion, extracellular matrix composition and local immune function [86]. Mammalian cells express three isotypes of transforming growth factor-β, which include TGFβ1, TGFβ2 and TGFβ3. In addition, there are three receptors, TβR-1, TβR-2 and TβR-3 [87,88], although only TβR-2 is over-expressed in cancer. Other members of the TGF superfamily include activin/inhibin and bone morphogenic proteins (BMPs) [87,88]. As with all the potential markers reviewed thus far, the evidence regarding the prognostic potential of the TGFβ family is conflicting (Table 9) [39,42,89,90]. Some studies have reported that the presence of TGFβ1 is associated with better survival, whilst others have reported the opposite.

4.2. Epidermal growth factor (EGF) superfamily and receptors

Epidermal growth factor is a polypeptide that induces proliferation of epidermal tissues when administered to animals. Many other members of the EGF family have since been described, which include transforming growth factor alpha (TGFα), heparin-binding EGF-like growth factor (HB-EGF), amphiregulin, betacellulin, epiregulin, neuregulins and cripto [91–95].

The EGF receptor is a 170 kDa protein on the cell surface. The EGF receptor is known as EGFR-1 (HER-1) and is closely related to several other receptors including HER 2 (c-erbB2), HER 3 (c-erbB3) and HER-4 (c-erbB4). All receptors are characterised by their tyrosine kinase activity [86].

There has been extensive work undertaken on the relationship between growth factors and growth factor receptors and survival following resection of pancreatic cancer, summarised in Tables 9 and 10 [11,39,42,68,89,90,96–114]. Over-expression of EGF is common, appearing on average in 50% of pancreatic cancer specimens. Most of the evidence points to increased expression of EGF correlating with advanced tumour stage. Concomitant over-expression of both EGF and its receptor would appear to give more reliable prognostic information than either factor alone. Expression of the other growth factor receptors appears to vary widely between studies (Table 10). Although most studies have shown a link between receptor expression and tumour stage/grade, this does not seem to be reflected in overall survival figures.

4.3. Fibroblast growth factors (FGFs)

Fibroblast growth factors comprise a family of 20 molecules with a wide range of biological factors. Among their actions is the ability strongly to stimulate angiogenesis; however, they are also involved in cell differentiation, tissue regeneration, repair and cell

Table 9
Growth factor and growth factor receptors and outcome in pancreatic cancer

Study	Year	Patients	Marker	Method	Number of patients with increased expression (%)	Correlation with survival	Correlation with tumour stage	Significance
Hashimoto *et al.* [39]	2001	62	TGFβ 1	IHC	45	TGFβ positive tumours found to have better prognosis	–	$P < 0.05$
Ito *et al.* [97]	2001	40	HB-EGF	IHC	55	–	Expression associated in well-differentiated, lower stage tumours without lymph node metastases	–
Wagner *et al.* [89]	1999	42	Tβ R-2 (receptor)	IHC	45	Higher levels of Tβ R-2 resulted in shorter survival times	–	$P < 0.05$
Dong *et al.* [96]	1998	57	EGF EGFR	IHC	73.7 68.4	Co-expression of EGF and EGFR correlated to significantly shorter survival (17.2 months longer)	No correlation No correlation	$P = 0.02$
Coppola *et al.* [42]	1998	42	TGFβ 1	IHC	31	TGFβ 1 expression associated with better survival	TGFβ 1 expression higher in lower grade tumours	$P < 0.05$
Gansauge *et al.* [68]	1998	82	EGF EGFR	IHC	30.4 46	No correlation	No correlation	–
Uegaki *et al.* [98]	1997	60 and 26 metastatic lesions	EGF EGFR	IHC	28 in primary lesions and 46% in metastatic lesions 43% in primary lesions and 46% in metastatic lesions	Co-expression of EGF and EGFR correlated with significantly shorter survival	EGF expression higher in metastatic lesions –	$P = 0.07$
Friess *et al.* [90]	1993	60	TGFβ 1 TGFβ 2 TGFβ 3	IHC and Northern Blot	47 42 40	Expression of TGFβ isoforms associated with decreased post-operative survival	No correlation Presence of TGFβ 2 correlated with advanced tumour stage No correlation	$P < 0.05$ $P < 0.001$ $P < 0.01$
Yamanaka *et al.* [101]	1993	87	EGF EGFR TGFα	IHC	46 43 54	Expression correlated with decreased post-operative survival	Expression associated with increased tumour size and advanced tumour stage	$P < 0.05$
Yamanaka [100]	1992	25	EGF EGFR c-erbB2	IHC	72 31 28	Co-expression of proteins associated with shorter survival	Expression associated with local invasion	$P < 0.05$

EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; IHC, immunohistochemical method.

Table 10
Growth factor and growth factor receptors and outcome in pancreatic cancer

Study	Year	Patients	Marker	Method	Number of patients with increased expression	Correlation with survival	Correlation with tumour stage	Significance
Ueda *et al.* [102]	2004	76	EGFR	IHC	–	Cytoplasmic EGFR associated with shorter survival	Cytoplasmic EGFR more frequent in higher grade tumours	$P < 0.001$
			HER-2	IHC	–	No correlation	No correlation	–
Tamiolakis *et al.* [103]	2004	100	HER-2	IHC	21%	No correlation	No correlation	–
Tobita *et al.* [104]	2003	77	EGFR	IHC	Diffuse expression in 32.5%, with increased in 9.1%	–	Correlation with tumour stage, grade and TNM classification	$P < 0.01$
Zhang *et al.*	2002	36	EGFR TGFβ 1 c-erbB2	IHC	7.7% 44.4% 7.7%	– – –	Co-expression (27.8%) was correlated to histological grade and clinical stage of tumours	$P < 0.01$
Koka *et al.* [105]	2002	308	HER-2	IHC	16% positive with 33% over-expression	No correlation	No correlation	–
Thybusch-Bernhardt *et al.* [107]	2001	24	HER-1 HER-2 HER-3 HER-4	IHC	33% 25% 50% 37%	– – – –	HER-1 and HER-2 correlated with advanced tumour stage No correlation HER-4 only found in non-metastatic tumours	$P = 0.07$
Safran *et al.* [108]	2001	154	HER-2	IHC	21%	–	No correlation with tumour stage or resectability	–
Novotny *et al.* [109]	2001	57	c-erbB2	IHC	19.6%	No correlation	No correlation	–
Kawesha *et al.* [11]	2000	157	c-erbB2 c-erbB3	IHC	33% 57%	No correlation No correlation	No correlation No correlation	– –
Graber *et al.* [110]	1999	75	c-erbB4 mRNA	PCR and Northern Blot	81.3%	No correlation	Increased mRNA expression associated with higher stage tumours and metastatic spread	$P < 0.01$
Kuniyasi *et al.* [114]	1999	22	EGF-R	IHC	–	No correlation	No correlation	–
Dugan *et al.* [111]	1997	68	HER-2	IHC	58%	No correlation	Higher HER-2 expression in well-differentiated tumours when compared to poorly differentiated (62% versus 19%)	–
Okada *et al.* [112]	1995	100	Serum and tissue c-erbB2 protein	IHC	34% positive in serum and 28% in tissue	Serum c-erbB2 levels correlated with shorter survival	Serum and tissue c-erbB2 levels correlated with presence of metastasis	$P < 0.01$
Lei *et al.* [113]	1995	21	HER-2	IHC	47.6%	Ovexpression linked to shorter survival (15months *versus* 5.2 months)	–	$P < 0.01$
Friess *et al.* [99]	1995	58	erbB3 mRNA	PCR and Northern Blot	46.6%	Expression associated with shorter survival	Expression associated with advanced tumour stage	$P < 0.01$

EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; IHC, immunohistochemical method.

migration. The two most extensively studied FGFs are FGF-1 (also known as acidic fibroblast growth factor, aFGF) and FGF-2 (basic fibroblast growth factor, bFGF) [115,116]. The bFGF receptor has been reported to correlate with lymph node metastasis, tumour stage and retroperitoneal invasion. In addition, low bFGF receptor expression has been associated with longer post-operative survival [117]. The same study and another report by Kuniyasu and colleagues found no correlation between bFGF and either tumour stage or survival [114]. A further study by Yamanaka and colleagues found that both aFGF and bFGF correlated with tumour stage, but only bFGF correlated with patient survival [118].

5. Extracellular matrix and tumour–stroma interaction

The poor prognosis of pancreatic cancer is dependent on its invasive and metastatic capabilities. Invasion of tumour cells into surrounding tissues relies on loosening of cell–cell adhesion, invasion of the basement membrane, and disassembly of the extracellular matrix (ECM). These conditions rely on a complex interaction between the expression and activity of enzymes, such as matrix-metalloproteinases, and the integrity of proteins comprising the ECM.

5.1. Matrix-metalloproteinases (MMPs)

MMPs are a family of zinc-containing proteolytic enzymes that break down extracellular matrix proteins [58]. One of the first steps of cancer invasion is the degradation of the basement membrane. This is predominately comprised of type IV collagen, and the two main types of type IV collagenases are MMP-2 (gelatinase A) and MMP-9 (gelatinase B) [119]. MMPs are broadly classified by their substrate specificity, and hence MMP-2 and MMP-9 are known as gelatinases, other groups include the collagenases (MMP-1, MMP-8 and MMP-13) and the stromelysins (MMP-3, MMP-10, MMP-11 and MMP-19). MMP-7 (also known as matrilysin), MMP-12 and MP-18 are known as 'ungrouped'. Recently, a group of membrane-bound MMPs has been described (MMP-14, MMP-15, MMP-16 and MMP-17, also known as MT-MMP1, MT-MMP2, MT-MMP3 and MTMMP4) [119]. MMP activity is tightly regulated by cytokines, growth factors and oncogenes. In addition, inhibitors of MMP known as TIMPs exist; four have been characterised so far and are known as TIMP-1, TIMP-2, TIMP-3 and TIMP-4.

In addition to using immunohistochemistry to assess MMP expression in tissues, zymography can be employed as a measure of MMP activation. Table 11 summarises data regarding MMP activation and expression and survival from pancreatic cancer [114,120–126]. Most studies have found that increased MMP expression correlates with poorer prognosis, shorter survival time and/or the presence of local invasion or distant metastases. On an individual assessment, MMP-7 or matrilysin has consistently been reported to have a negative impact on survival, however for the other MMPs conflicting data does exist. It may be that looking at co-expression of combination of MMPs may allow for more consistent prediction of survival, rather than looking at one MMP in isolation.

5.2. Urokinase plasminogen activator (uPA)

The enzyme uPA converts the inactive plasminogen into the potent protease plasmin. In addition to degradation of fibrin, type IV collagen, fibronectin and laminin, plasmin also activates precursors of MMPs, hence this cascade leads to extensive degradation of the ECM. At present, both uPA and its receptor appear to be increased in pancreatic cancer tissue, and this over-expression of the two results in shorter survival (Table 12) [120,127].

5.3. Cathepsins

The term cathepsins includes serine, cysteine and aspartyl-type proteases. The cathepsins are intracellular proteases that function in terminal protein degradation by lysosomes. In addition, cathepsins also play roles in bone remodelling, epidermal haemostasis and antigen presentation [128]. Cathepsins are also over-expressed in malignant cells, either on the cell surface or as secretory proteins. Although cathepsins appear to be linked in the malignant progression of pancreatic cancer, at present, there are insufficient data on their possible application as prognostic biomarkers (Table 12) [129,130].

5.4. Heparanase

Heparanase is a relatively new addition to the ECM degradation enzymes. This enzyme cleaves heparin sulphate proteoglycans (HSPG). Furthermore, heparanase also acts to release growth factors such as bFGF and heparin sulphate, and so stimulates growth and angiogenesis. Early reports are encouraging so far, linking heparanase expression to decreased survival post-resection (Table 12) [131–133].

5.5. Laminins, CD44 variants, and the integrins

Members of the laminin family of glycoproteins are major constituents of basement membranes. Laminins have one heavy α chain and two light chains designated β and γ. So far, one study has looked at laminin $\gamma 2$ expression in patients with pancreatic cancer and found

Table 11
Matrix-metalloproteinases (MMPs) and survival in pancreatic cancer

Study	Year	Patients	Marker investigated	Method	Percentage of patients with positive expression (%)	Correlation with survival	Correlation with tumour stage	Significance
Harvey *et al.* [120]	2003	27	MMP-9	IHC	37	Trend towards negative correlation with survival	No correlation	NS
Nakamura *et al.* [121]	2002	39	MMP-7	IHC	64% positive	Shorter survival time in MMP 7 positive patients	Correlated with lymph node metastases and infiltrating growth pattern	–
Yammamoto *et al.* [122]	2001	70	MMP-1	IHC	70	No correlation with survival	No correlation	–
			MMP-2		94			
			MMP-3		20			
			MMP-7		56	Independent prognostic factor for survival	Correlation with TNM staging ($P < 0.0001$)	$P = 0.022$
			MMP-9		93	No correlation with survival	No correlation	–
			MT-MMP1		67			
			TIMP-1		87			
			TIMP-2		71			
Maatta *et al.* [123]	2000	35	MMP-2	IHC and Northern Blot analysis and ISH	85% RNA+	No correlation	No correlation	NS
			MMP-9		91% RNA+	No correlation		
			MT-MMP1		97% RNA+	Increased mRNA expression associated with poorer prognosis		
Gong *et al.* [124]	2000	15	MMP-2	Northern Blot analysis and ISH	58.4	No correlation	Higher expression of MMP and lower expression of TIMP-1 found in poorly differentiated tumours ($P < 0.05$)	–
			MMP-9		56.5			
			TIMP-1		82.3			
Ito *et al.* [125]	1999	46	MMP-1	IHC	72	MMP-1 positive patients had significantly poorer prognosis that MMP-1 negative	No correlation	$P < 0.05$
Kuniyasu *et al.* [114]	1999	22	MMP-2	Colorimetric *in situ* mRNA hybridisation	86	No correlation	No correlation	–
			MMP-9		91	MMP-9 expression correlated with overall survival	No correlation	$P = 0.0249$
Koshiba *et al.* [126]	1998	33	MMP-2	Zymography and Western Blotting	100% expression	Activation ratio of associated with post-resection recurrence at 6 months	Activation ratio higher in tumours with lymph node metastases and distant metastases	$P < 0.05$
			MMP-9		33	No correlation	No correlation	–

ISH, *in-situ* hybridisation; IHC, immunohistochemical method.

Table 12
ECM proteins and proteases and survival in pancreatic cancer

Study	Year	Patients	Marker investigated	Method	Number of patients with detected expression (%)	Correlation with survival	Correlation with tumour stage or invasion	Significance
Harvey *et al.* [120]	2003	27	uPA uPAR	ISH for mRNA	93% 52%	Over-expression associated with shorter survival	No correlation	NS
Cantero *et al.* [127]	1997	30	uPA uPAR	IHC and Northern Blot	–	Co-expression of uPA and uPAR had a shorter survival than patients with one factor or no factors over-expressed	No correlation	$P < 0.002$
Niedergethmann *et al.* [129]	2000	29	Cathepsin B	IHC	96.5%	Strength of expression correlated with survival time after surgery	Degree of expression correlated with invasion of perineural space	$P = 0.0002$
			Cathepsin L		84.2%	Strength of expression correlated with survival time after surgery	No correlation	$P = 0.0001$
Tumminello *et al.* [13]	1996	34	Cathepsin D Cathepsin B Cathepsin L	IHC	–	No correlation	No correlation	–
Rohloff *et al.* [131]	2002	50	Heparanase	IHC	78% some + expression	Negative correlation between Heparanase and post-operative survival	No correlation	$P < 0.01$
Kim *et al.* [132]		89	Heparanase receptor	ISH	78%	Receptor expression in early stage tumours correlated with decreased survival	No correlation	–
Koliopanos *et al.* [133]	2001	33	Heparanase	PCR for RNA	–	Negative correlation between Heparanase and post-operative survival	No correlation	$P < 0.01$
Takahashi *et al.* [134]	2001	48	Laminin $\gamma 2$	IHC	Cytoplasmic expression 54.2% and basement membrane expression 45.8%	Cytoplasmic expression strongest predictive factor for poor overall survival	Cytoplasmic expression associated with occurrence of post-operative liver metastases. Basement membrane expression associated with tumour differentiation.	$P = 0.0161$
Gotoda *et al.* [135]	1998	42	CD44v6	IHC	50%	Correlated with decreased survival	No correlation	$P = 0.0160$
			CD44v2		38%	Correlated with decreased survival	Correlated with vessel invasion	$P = 0.0125$
Gansauge *et al.* [136]	1997	93	CD44s CD44v6	IHC	–	No correlation Decreased serum CD44v6 correlated with reduced survival	– –	– $P < 0.0005$

(*continued on next page*)

Table 12 (*continued*)

Study	Year	Patients	Marker investigated	Method	Number of patients with detected expression (%)	Correlation with survival	Correlation with tumour stage or invasion	Significance
Tomaszewska *et al.* [137]	1999	12	CD44s CD44v6	IHC	– –	– –	No correlation Higher expression in higher grades of tumour	– –
Bottger *et al.* [138]	1998	40	CD44v4, v5, v6, v7, v10	IHC	–	Over-expression associated with worse prognosis	–	NS
Sawai *et al.* [140]	2003	20	α5β1 and α6β1	IHC	–	Strong expression of α 6, or weak expression of α 5 associated with poor prognosis	Significant association with presence of liver metastases and advanced stage	$P < 0.005$
Hosotani *et al.* [139]	2002	50	α5β3 integrin	IHC	58%	No correlation with survival	Significantly higher expression in patients with lymph node metastases	–
Bottger *et al.* [141]	1999	42	β1 integrin	IHC	–	No correlation	No correlation	–

IHC, immunohistochemical method; PCR, polymerase chain reaction.

it to hold significant prognostic information (Table 12) [134]. CD44 is a heavily glycosylated cell surface molecule which is involved in cell–cell and cell–matrix interaction. CD44 has several functions, which includes extracellular matrix cell adhesion. CD44 is encoded by a single complex gene, producing a constitutively expressed protein known as CD44s from 10 exons, and a large array of protein isoforms, known as CDD44v, produced from alternative slicing of the remaining exons. The best studied, in relation to pancreatic cancer, is CD44v6, which has shown a statistically significant correlation with decreased survival in most studies [135–138] (Table 12).

Cell adhesion to the ECM is, at least in part, mediated by the integrin family of transmembrane receptor proteins. These consist of at least 16 α and 8 β subunits, which form numerous heterodimers, each with distinct adhesion properties. Limited clinical data on integrins and survival from pancreatic cancer exist, and more work is needed in this area (Table 12) [139–141].

5.6. The cadherin/catenin complex

E-Cadherin and its associated cytoplasmic catenins are important mediators of cell–cell adhesion; here they are discussed separately from tumour–stroma interaction proteins, because of their additional role in intracellular signalling. E-Cadherin is a membrane-bound protein, whose extracellular domain interacts with neighbouring cells to form tight cell–cell adhesions. β-Catenin directly connects the intracellular domain of E-cadherin to α-catenin, which connects to the actin cytoskeleton of the cell. β-Catenin is a 92 kDa protein, which has a dual role as an intracellular adhesion molecule and also as a key downstream effector of the Wnt signalling pathway [142]. *Cyclin D1* has recently been identified as a target gene of β-catenin in colorectal, breast and pancreatic cancer, and hence over-expression of *cyclin D1* could be driven by nuclear accumulation of β-catenin [69].

Most studies thus far suggest that dysregulation of β-catenin (nuclear/cytoplasmic accumulation, with loss of membranous expression) and reduced expression of E-cadherin negatively affects survival or correlates with deleterious clinicopathological features in patients with pancreatic tumours (Table 13) [69,70,143–146].

6. Angiogenesis

Previous research has shown that the proliferative index of tumours decreases with increasing distance from the nearest capillary blood vessel and that rapid exponential growth of tumours is dependent on vascularisation of the tumour mass [147–149]. Without angiogenesis, tumours are limited in size to the distance

Table 13
The cadherin/catenin complex and pancreatic cancer survival

Study	Year	Patients	Marker	Method	Correlation with survival	Correlation with tumour stage	Significance
Julkunen *et al.* [143]	2003	36	α-Catenin	IHC	Trend with lower survival	Significant correlation with tumour grade	–
			β-Catenin		β-Catenin demonstrated to be an independent prognostic factor		
			χ-Catenin		Trend with lower survival	–	
Li *et al.* [70]	2003	47	β-Catenin	IHC	Over-expression of β-Catenin linked to lower 1-year survival rate	Over-expression of β-Catenin correlated to presence of metastases	$P = 0.05$
Lowy *et al.* [144]	2003	57	β-Catenin	IHC	–	Reduced membranous expression correlated with loss of tumour differentiation	$P = 0.05$
Joo *et al.* [145]	2002	30	α-Catenin	IHC	–	Correlated with tumour differentiation. Reduced expression correlated with stage, lymph node involvement and tumour differentiation	$P = 0.05$
			β-Catenin		–		
			E-Cadherin		–		
Karayiannakis *et al.* [146]	2001	43	α-Catenin	IHC	Independent prognostic factor for survival	Correlation with disease stage, lymph node and distant metastases	$P = 0.05$
			β-Catenin		Associated with poor prognosis	Correlation with lymph nodded metastases	
			γ-Catenin			Correlation with disease stage, lymph node and distant metastases	
			E-Cadherin		Independent prognostic factor for survival	–	
Qiao *et al.* [69]	2001	43	β-Catenin	IHC	Cytoplasmic over-expression of β-Catenin associated with reduced 1-year survival	–	$P < 0.01$

IHC, immunohistochemical method.

Table 14
Angiogenic factors and outcome in pancreatic cancer

Study	Year	Patients	Angiogenic factor	Method	% of positive staining	Correlation with survival	Expression and tumour stage/grade ± efficacy of chemotherapy	Significance
Kuwahara *et al.* [151]	2003	55	VEGF FGF PD-ECGF	IHC	70.8 60.9 57.2	High expression of VEGF and FGF correlated with significantly shorter survival	No correlation	$P < 0.05$
Karayiannakis *et al.* [151]	2003	58	Serum VEGF	ELISA	–	Elevated serum VEGF significant prognostic factor for survival	Correlation with higher disease stage and presence of distant metastases ($P = 0.001$)	$P = 0.002$
Buchler *et al.* [153]	2002	24	VEGFR-I VEGFR-II	IHC and Northern Blot	70.8 62.5	No correlation Associated with poor survival	No correlation Correlated with poor tumour differentiation	– $P < 0.05$
Tobita *et al.* [166]	2002	77	TSP-1	IHC	–	Reduced TSP-1 expression associated with poor prognosis	TSP-1 expression correlated to lymphatic, venous invasion and tumour stage	$P < 0.01$
Niedergethmann *et al.* [154]	2002	72	VEGF	ISH for mRNA	88.6	VEGF expression found to be an independent prognostic marker for cancer recurrence 8 months after curative surgery	–	$P = 0.003$
Fujioka *et al.* [155]	2001	104	VEGF TP bFGF	IHC	– – –	No correlation Local recurrence more common in patients with positive staining for TP and bFGF	No correlation Hepatic metastases more frequent in patients with cytoplasmic expression of TP and bFGF	– $P < 0.05$
Minari *et al.* [161]	2001	66 primary lesions, 46 nodal, 36 metastatic	TP	IHC	71, 46 and 53, respectively	No correlation in primary tumours only. In patients with nodal involvement TP associated with poorer prognosis.	No impact on efficacy of chemotherapy	$P < 0.05$
Seo *et al.* [156]	2000	142	VEGF	IHC	93	High or moderate VEGF expression associated with shorter survival	High VEGF expression associated with liver metastases ($P = 0.010$)	$P = 0.05$
Ikeda *et al.* [157]	1999	40	VEGF	IHC and PCR for gene expression	67.5	Positive VEGF expression associated with shorter survival, VEGF shown to be independent prognostic factor by COX analysis	Positive correlation with histopathological grading ($P = 0.0058$)	$P = 0.0443$
			PD-ECGF		75	Positive PD-ECGF expression associated with shorter survival	No correlation	$P = 0.040$

Fujimoto *et al.* [158]	1998	50	VEGF	IHC	56	No correlation	Correlation with microvessel density (MVD), but no correlation with clinicopathological variables	–
			PF-ECGF		32	Correlated with poor survival		$P = 0.011$
Takao *et al.* [162]	1998	54	TP	IHC	59	Significant correlation with poor survival	Correlation with extrapancreatic neural plexus invasion and presence of post-operative hepatic metastases ($P < 0.05$)	$P = 0.013$
Ellis *et al.* [159]	1998	22	VEGF	IHC	–	No correlation	No correlation	–
Itakura *et al.* [160]	1997	75	VEGF	IHC and ISH	64	No correlation with survival	VEGF expression associated with enhanced local spread and tumour size	–
Shimoyama *et al.* [167]	1996	37	Angiogenin	ISH	–	High serum levels of serum angiogenin associated with poorer survival	No correlation	$P < 0.05$

TP, thymidine phosphorylase or PD-ECG; VEGF, vascular endothelial derived growth factor; IHC, immunohistochemical method; PCR, polymerase chain reaction; ISH, *in-situ* hybridisation; FGF, fibroblast growth factors.

that oxygen can diffuse, namely 1–2 mm. Furthermore, increased vascularity not only allows an expansion in tumour size, it leads to a greater probability of haematogenous embolisation of the tumour and so metastatic spread. Tumour foci in distant organs are also reliant on angiogenesis to establish an independent blood supply. Hence, the expression of pro-angiogenic factors and pancreatic cancer survival has received considerable attention.

6.1. Vascular endothelial derived growth factor (VEGF)

The VEGF family consists of 6 different cytokines, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placenta growth factor (PIGF) [2,150]. VEGF-A is the best studied of this group and consists of a sulphide-bonded dimeric glycoprotein of 34–45 kDa, coded on chromosome 6p [2,150]. Five isoforms of VEGF-A have been described, but in pancreatic cancer the predominant species are VEGF165 and VEGF121. VEGF is both chemotactic and mitogenic for endothelial cells, and acts to increase the permeability of the vascular endothelium. The evidence for the potential of VEGF, and its receptor, being an important prognostic marker in pancreatic cancer is compelling, with most studies showing it to be correlated to survival or to adverse clinicopathological variables (Table 14) [151–160].

6.2. Platelet-derived endothelial growth factor (PD-ECGF)/thymidine phosphorylase

PD-ECGF or thymidine phosphorylase is a 55 kDa protein that stimulates chemotaxis of endothelia cells. Second to VEGF, PD-ECGF is one of the most commonly studied pro-angiogenic factors in pancreatic cancer. Of the six studies reported thus far on PD-ECGF, all bar one have found over-expression to be linked to poor survival or with adverse tumour characteristics (Table 14) [151,155,157,158,161,162].

6.3. Other angiogenic biomarkers

Thrombospondin (TSP) inhibits angiogenesis and is released by platelets in the presence of thrombin [163–165]. Of the five subtypes of TSP, TSP-1 and TSP-2 have been the most extensively studied in relation to the inhibition of angiogenesis. TSP-1 is a 450 kDa glycoprotein that has been found to be highly expressed in the stroma surrounding tumour cells in human pancreatic cancer [163–165] and to correlate inversely with microvessel density [163–165]. High expression of TSP-1 is also a favourable prognostic indicator in pancreatic ductal carcinoma (Table 14) [166].

Angiogenin is an inducer of vascularisation. Angiogenin binds to actin on endothelial cells, and results in activation of several protease cascades [58]. Angiogenin

mRNA levels in the serum have been associated with shorter survival in patients with pancreatic tumours (Table 14) [167].

7. Conclusion

A bewildering number of biological markers of pancreatic cancer progression are under evaluation, some with greater potential than others in their ability to predict survival in patients with pancreatic tumours. These markers have a number of possible applications, beyond their association with survival and clinicopathological characteristics of tumours; including the possibility to become targets for intervention. The future application of these biomarkers is yet to be fully realised. What is evident is that considerable work is needed to develop these markers into viable clinical tools. Until now, cancer research has progressed in a linear fashion, examining only one or two targets at a time. In this way, the true relationship and interplay between these molecular carcinogenic cascades may be lost. With the advent of gene microarray and mass spectrometry, this need no longer be the case. These powerful research tools allow us to measure and correlate multiple genetic and down-stream protein parameters for pancreatic tissue and surrogate tissue such as sera. Recent reports have already attempted to find a 'protein signature' that could be used to diagnose pancreatic tumours [168–170]. Although this work is still in its early stages, it may enable us to evaluate multiple biomarkers at the same time, and so achieve a reproducible and accurate prognostic model for patients with pancreatic tumours.

Conflict of interest statement

None declared.

References

1. Real FA. A 'catastrophic hypothesis' for pancreas cancer progression. *Gastroenterology* 2003, **124**, 1958–1964.
2. Duffy JP, Eibl G, Reber HA, *et al*. Influence of hypoxia and neoangiogenesis on the growth of pancreatic cancer. *Mol Cancer* 2003, **2**, 12.
3. Miller BA, Koloenel LN, Berstein L. *Raciallethnic patterns of cancer in the United States 1998–92. NIH Publication No. 96-4104*. Bethseda, MD, National Cancer Institute, 1996.
4. Niederhuber JE, Brennan MF, Menck HR. The National Cancer Data Base report on pancreatic cancer. *Cancer* 1995, **76**, 1671–1677.
5. Solcia E, Capella C, Kloppel G. *Tumours of the pancreas*. Washington, DC, Universities Associated for Research and Education in Pathology, 1995.
6. Wade TP, Halaby IA, Stapleton DR, *et al*. Population based analysis of treatment of pancreatic cancer and Whipple resection: Department of Defence Hospitals 1989–1994. *Surgery* 1996, **120**, 680–687.
7. Moore PS, Orlandini S, Zamboni G, *et al*. Pancreatic tumours: molecular pathways implicated in ductal cancer are involved in ampullary but not in exocrine nonductal or endocrine tumorigenesis. *Br J Cancer* 2001, **84**, 253–262.
8. Rozenblum E, Schutte M, Goggins M, *et al*. Tumour-suppressive pathways in pancreatic carcinoma. *Cancer Res* 1997, **57**, 1731–1734.
9. Hickman JA. Apoptosis induced by anticancer drugs. *Cancer Metast Rev* 1992, **11**, 121–139.
10. Naka T, Kobayashi M, Ashida K, *et al*. Aberrant p16INK4 expression related to clinical stage and prognosis in patients with pancreatic cancer. *Int J Oncol* 1998, **12**(5), 1111–1116.
11. Kawesha A, Ghaneh P, Andren-Sandberg A, *et al*. 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* 2000, **89**(6), 469–474.
12. Dong M, Nio Y, Yamasawa K, *et al*. p53 alteration is not an independent prognostic indicator, but affects the efficacy of adjuvant chemotherapy in human pancreatic cancer. *J Surg Oncol* 2003, **82**, 111–120.
13. Nio Y, Dong M, Iguchi C, *et al*. Expression of Bcl-2 and p53 protein in resectable invasive ductal carcinoma of the pancreas: effects on clinical outcome and efficacy of adjuvant chemotherapy. *J Surg Oncol* 2001, **76**, 188–196.
14. Hu YX, Watanabe H, Ohtsubo K, *et al*. Bcl-2 expression related to altered p53 protein and its impact on the progression of human pancreatic carcinoma. *Br J Cancer* 1999, **80**, 1075–1079.
15. Lundin J, Nordling S, von Boguslawsky K, *et al*. Prognostic value of immunohistochemical expression of p53 in patients with pancreatic cancer. *Oncology* 1996, **53**(2), 104–111.
16. Nio Y, Dong M, Uegaki K, *et al*. p53 expression affects the efficacy of adjuvant chemotherapy after resection of invasive ductal carcinoma of the pancreas. *Anticancer Res* 1998, **18**(5B), 3773–3779.
17. Nio Y, Dong M, Uegaki K, *et al*. Comparative significance of p53 and WAF/1-p21 expression on the efficacy of adjuvant chemotherapy for resectable invasive ductal carcinoma of the pancreas. *Pancreas* 1999, **18**(2), 117–126.
18. Dong M, Nio Y, Tamura K, *et al*. Ki-ras point mutation and p53 expression in human pancreatic cancer: a comparative study among Chinese, Japanese and Western patients. *Cancer Epidemiol Biomarkers Prev* 2000, **9**, 279–284.
19. Dergham ST, Dugan MC, Kucway R, *et al*. Prevalence and clinical significance of combined K-ras mutation and p53 aberration in pancreatic adenocarcinoma. *Int J Pancreatol* 1997, **21**(2), 127–143.
20. Yokoyama M, Yamanaka Y, Friess H, *et al*. p53 expression in human pancreatic cancer correlates with enhanced biological aggressiveness. *Anticancer Res* 1994, **14**(6B), 2477–2483.
21. Nakamori S, Yashima K, Murakami Y, *et al*. Association of p53 gene mutations with short survival in pancreatic adenocarcinoma. *Jpn J Cancer Res* 1995, **86**(2), 174–181.
22. Zhang SY, Ruggeri B, Agarwal P, *et al*. Immunohistochemical analysis of p53 expression in human pancreatic carcinomas. *Arch Pathol Lab Med* 1994, **118**(2), 150–154.
23. DiGiuseppe JA, Hruban RH, Goodman SN, *et al*. Overexpression of p53 protein in adenocarcinoma of the pancreas. *Am J Clin Pathol* 1994, **101**, 684–688.
24. Ruggeri BA, Huang L, Berger D, *et al*. Molecular pathology of primary and metastatic ductal pancreatic lesions: analyses of mutations and expression of the p53, mdm-2, and p21/WAF-1 genes in sporadic and familial lesions. *Cancer* 1997, **79**(4), 700–716.

25. Biankin AV, Morey AL, Lee C, *et al*. DPC4/Smad4 expression and outcome in pancreatic ductal adenocarcinoma. *J Clin Oncol* 2002, **20**, 4531–4542.
26. Campani D, Boggi U, Cecchetti D, *et al*. p53 overexpression in lymph node metastases predicts clinical outcome in ductal pancreatic cancer. *Pancreas* 1999, **19**, 26–32.
27. Ohshio G, Suwa H, Immamura T, *et al*. An immunohistochemical study of blc-2 and p53 protein expression in pancreatic carcinomas. *Scand J Gastroenterol* 1998, **b33**, 535–539.
28. Makinen K, Hakala T, Lipponen P, *et al*. Clinical contribution of bcl-2, p53 and Ki-67 proteins in pancreatic ductal adenocarcinoma. *Anticancer Res* 1998, **18**, 615–618.
29. Mueller H, Eppenberger U. The dual role of mutant p53 protein in chemosensitivity of human cancers. *Anticancer Res* 1996, **16**(6B), 3845–3848.
30. Petty RD, Cree IA, Sutherland LA. Expression of the p53 tumour suppressor gene product is a determinant of chemosensitivity. *Biochem Biophys Res Comm* 1994, **199**, 264–270.
31. Fan S, Smith ML, Rivet DJ. Disruption of p53 function sensitizes breast cancer MCF-7 cells to cisplatin and pentoxifylline. *Cancer Res* 1995, **55**, 1649–1654.
32. Serrano M, Hannon GJ, Beach D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature* 1993, **366**, 704–707.
33. Ohtsubo K, Watanabe H, Yamaguchi Y, *et al*. Abnormalities of tumor suppressor gene p16 in pancreatic carcinoma: immunohistochemical and genetic findings compared with clinicopathological parameters. *J Gastroenterol* 2003, **38**(7), 663–671.
34. Gerdes B, Ramaswamy A, Ziegler A, *et al*. p16INK4a is a prognostic marker in resected ductal pancreatic cancer: an analysis of p16INK4a, p53, MDM2, an Rb. *Ann Surg* 2002, **235**(1), 51–59.
35. Hu YX, Watanabe H, Ohtsubo K, *et al*. Frequent loss of p16 expression and its correlation with clinicopathological parameters in pancreatic carcinoma. *Clin Cancer Res* 1997, **3**(9), 1473–1477.
36. Sasaki S, Yamamoto H, Kaneto H, *et al*. Differential roles of alterations of p53, p16, and SMAD4 expression in the progression of intraductal papillary-mucinous tumors of the pancreas. *Oncol Rep* 2003, **10**(1), 21–25.
37. Weiss RH. p21Waf1/Cip1 as a therapeutic target in breast and other cancers. *Cancer Cell* 2003, **4**(6), 425–429.
38. Xiong Y, Hannon GJ, Zhang H, *et al*. p21 is a universal inhibitor of cyclin kinases. *Nature* 1993, **366**(6456), 701–704.
39. Hashimoto Y, Nio Y, Sumi S, *et al*. Correlation between TGF-beta 1 and p21 (WAFI/CIP1) expression and prognosis in resectable invasive ductal carcinoma of the pancreas. *Pancreas* 2001, **22**, 341–371.
40. Song MM, Nio Y, Dong M, *et al*. Comparison of K-ras mutation at codon 12 and p21 expression in pancreatic cancer between Japanese and Chinese patients. *J Surgical Oncol* 2000, **75**, 176–185.
41. Ahrendt SA, Brown HM, Komorowski RA, *et al*. p21WAF1 expression is associated with improved survival after adjuvant chemoradiation for pancreatic cancer. *Surgery* 2000, **128**, 520–530.
42. Coppola D, Lu L, Frehauf JP, *et al*. Analysis of p53, p21WAF1, and TGF-beta1 in human ductal adenocarcinoma of the pancreas: TGF-beta 1 protein expression predicts longer survival. *Am J Clin Pathol* 1998, **110**, 16–23.
43. Song MM, Nio Y, Sato Y, *et al*. Clinicopathological significance of K-ras point mutation and expression in benign and malignant exocrine tumours of the human pancreas. *Int J Pancreatology* 1996, **20**, 85–93.
44. Yamaguchi K, Enjoji M. Carcinoma of the pancreas: a clinicopathologic study of 96 cases with immunohistological observations. *Jap J Clin Oncol* 1989, **19**, 14–22.
45. Dergham ST, Dugan MC, Joshi US, *et al*. The clinical significance of p21(WAF1/CIP-1) and p53 expression in pancreatic adenocarcinoma. *Cancer* 1997, **80**(3), 372–381.
46. Hahn SA, Schutte M, Hoque AT, *et al*. DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. *Science* 1996, **271**(5247), 350–353.
47. Tang ZH, Zou SQ, Hao YH, *et al*. The relationship between loss expression of DPC4/Smad4 gene and carcinogenesis of pancreatobiliary carcinoma. *Hepatobiliary Pancreat Dis Int* 2002, **1**(4), 624–629.
48. Liu F. SMAD4/DPC4 and pancreatic cancer survival. *Clin Cancer Res* 2001, **7**, 3853–3856.
49. Schwarte-Waldhoff I, Schmiegel W. Smad4 transcriptional pathways and angiogenesis. *Int J Gastrointest Cancer* 2002, **31**(1–3), 47–59.
50. Hua Z, Zhang YC, Hu XM, Jia ZG. Loss of DPC4 expression and its correlation with clinicopathological parameters in pancreatic carcinoma. *World J Gastroenterol* 2003, **9**, 2764–2767.
51. Tascilar M, Skinner HG, Rosty C, *et al*. The SMAD4 protein and prognosis of pancreatic ductal adenocarcinoma. *Clin Cancer Res* 2001, **7**, 4115–41121.
52. Lu C, Morita S, Ishibashi T, *et al*. Loss of p27-kip1 expression independently predicts poor prognosis for patients with resectable pancreatic adenocarcinoma. *Cancer* 1999, **85**, 1250–1260.
53. Juuti A, Nordling S, Louhimo J, *et al*. Loss of p27 expression is associated with poor prognosis in stage I–II pancreatic cancer. *Oncology* 2003, **65**, 371–377.
54. Feakins RM, Ghaffar AJ. p27 Kip expression is reduced in pancreatic carcinoma but of limited prognostic value. *Hum Pathol* 2003, **34**, 385–390.
55. Grunewald K, Lyons J, Frohlich A. High frequency of Ki-ras codon 12 mutations in pancreatic adenocarcinomas. *Int J Cancer* 1989, **43**, 1037–1041.
56. Smits V, Boot A, Smit A. K-ras mutations occur very frequently in pancreatic adenocarcinomas. *Nucl Acids Res* 1988, **16**, 7773–7782.
57. Almoguera C, Shibatad KF. Most human carcinomas of the exocrine pancreas contain mutant K-ras genes. *Cell* 1988, **53**, 549–553.
58. Ghaneh P, Kawesha A, Evans JD, *et al*. Molecular prognostic markers in pancreatic cancer. *J Hepatobiliary Pancreat Surg* 2002, **9**, 1–11.
59. Castells A, Puig P, Mora J, *et al*. K-ras mutations in DNA extracted from the plasma of patients with pancreatic carcinoma: diagnostic utility and prognostic significance. *J Clin Oncol* 1999, **17**, 578–584.
60. Allison DC, Piantadosi S, Hruban RH, *et al*. DNA content and other factors associated with ten-year survival after resection of pancreatic carcinoma. *J Surg Oncol* 1998, **6**, 151–159.
61. Kitago M, Ueda M, Airura K, *et al*. Comparison of K-ras mutation distributions in intraductal papillary mucinous tumours and ductal adenocarcinoma of the pancreas. *Int J Cancer* 2004, **10**, 177–182.
62. Motojima K, Urano T, Nagata Y, *et al*. Mutations in the Kirsten-ras oncogene are common but lack correlation with prognosis and tumour stage in human pancreatic carcinoma. *Am J Gastroenterol* 1991, **86**, 1784–1788.
63. Finkelstein SD, Przygodzki RM, Pricolo VE, *et al*. K-ras-2 topographic genotyping of pancreatic adenocarcinoma. *Arch Surg* 1994, **129**, 367–372.
64. Hruban RH, Van Mansfield AD, Offerhaus G, *et al*. K-ras oncogene activation in adenocarcinoma of the human pancreas. A study of 82 carcinomas using a combination of mutant enriched chain reaction analysis and allele specific oligonucleotide hybridization. *Am J Pathol* 1993, **143**, 545–554.
65. Span M, Moerkerk PT, De Goeij AF, *et al*. A detailed analysis of K-ras mutations in relation to tumour progression and

survival in colorectal cancer patients. *Int J Cancer* 1996, **69**, 241–245.
66. Tetsu O, McCormick F. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature* 1999, **398**, 422–426.
67. Gansauge S, Gansuage F, Ramadani M, *et al*. Overexpression of cyclin D1 in human pancreatic carcinoma is associated with poor prognosis. *Cancer Res* 1997, **57**, 1634–1637.
68. Gansauge F, Gansauge S, Schmidt E, *et al*. Prognostic significance of molecular alterations in human pancreatic carcinoma: an immunological study. *Langebecks Arch Surg* 1998, **383**, 152–155.
69. Qiao Q, Ramadani M, Gansauge S, *et al*. Reduced membranous and ectopic cytoplasmic expression of beta-catenin correlate with cyclin D1 overexpression and poor prognosis in pancreatic cancer. *Int J Cancer* 2001, **95**, 194–197.
70. Li YJ, Ji XR. Relationship between the expression of beta-catenin, cyclin D1 and myc and the occurrence and biological behaviour of pancreatic cancer. *Zhonghua Bing Li Xue Za Zhi* 2003, **32**, 238–241.
71. Kornmann M, Ishiwata T, Itakura J, *et al*. Increased cyclin D1 in human pancreatic cancer is associated with decreased postoperative survival. *Oncology* 1998, **55**(4), 363–369.
72. Gross A, McDonnell TJ, Korsmeyer SJ. BCL-2 family members and the mitochondria in apoptosis. *Genes Dev* 1999, **13**, 1899–1911.
73. Greten FR, Weber CK, Greten TF, *et al*. Stat3 and NF-kB activation prevents apoptosis in pancreatic carcinogenesis. *Gastroenterology* 2002, **123**, 2052–2063.
74. Sinicrope FA, Evans DB, Leach SD, *et al*. bcl-2 and p53 expression in resectable pancreatic adenocarcinomas: association with clinical outcome. *Clin Cancer Res* 1996, **2**(12), 2015–2022.
75. Sun CY, Wang BL, Hu CQ, *et al*. Expression of the bcl-2 gene and its significance in human pancreatic cancer. *Hepatobiliary Pancreatic Dis Int* 2002, **1**, 306–308.
76. Nio Y, Iguchi C, Yamasawa K, *et al*. Apoptosis and expression of Bcl-2 and Bax proteins in invasive ductal carcinoma of the pancreas. *Pancreas* 2001, **22**, 230–239.
77. Evans JD, Cornford PA, Dodson A, *et al*. Detailed tissue expression of bcl-2, bax, bak and bcl-x in the normal human pancreas and chronic pancreatitis, ampullary and pancreatic ductal adenocarcinomas. *Pancreatology* 2001, **1**, 254–262.
78. Campani D, Esposito I, Boggi U, *et al*. Bcl-2 expression in pancreas development and pancreatic cancer progression. *J Pathol* 2001, **194**, 444–450.
79. Friess H, Lu Z, Graber HU, *et al*. bax, but not bcl-2, influences the prognosis of human pancreatic cancer. *Gut* 1998, **43**, 414–421.
80. Friess H, Lu Z, Andren-Sandberg A, *et al*. Moderate activation of the apoptosis inhibitor bcl-xL worsens prognosis in pancreatic cancer. *Ann Surg* 1998, **228**, 78–787.
81. Kami K, Doi R, Koizuma M, *et al*. Survivin expression is a prognostic marker in pancreatic cancer patients. *Surgery* 2004, **136**, 443–448.
82. Satoh K, Kaneko K, Hirota M, *et al*. Expression of survivin is correlated with cancer cell apoptosis and is involved in the development of human pancreatic duct cell tumours. *Cancer* 2001, **92**, 271–278.
83. Sarela AI, Verbeke CS, Ransdale J, *et al*. Expression of survivin, a novel inhibitor of apoptosis and cell cycle regulatory protein, in pancreatic adenocarcinoma. *Br J Cancer* 2002, **86**, 886–892.
84. Qiao JG, Zhang YQ, Yin YC, *et al*. Expression of survivin in pancreatic cancer and its correlation to expression of Bcl-2. *World J Gastroenterol* 2004, **10**, 2759–2761.
85. Asanuma K, Moriai R, Yajima T, *et al*. Survivin as a radioresistance factor in pancreatic cancer. *Jap J Cancer Res* 2000, **91**, 1204–1209.
86. Ozawa F, Friess H, Tempia-Caliera A, *et al*. Growth factors and their receptors in pancreatic cancer. *Teratogen, Carcinogen Mutagen* 2001, **21**, 27–44.
87. Massague J, Cheifetz S, Laiho M, *et al*. Transforming growth factor-beta. *Cancer Surv* 1992, **12**, 81–103.
88. Massague J. The transforming growth factor-beta family. *Ann Rev Cell Biol* 1990, **6**, 597–641.
89. Wagner M, Kleeff J, Friess H, *et al*. Enhanced expression of the type II transforming growth factor beta receptor is associated with decreased survival in human pancreatic cancer. *Pancreas* 1999, **19**, 370–376.
90. Friess H, Yamanaka Y, Buchler M, *et al*. Enhanced expression of transforming growth factor beta isoform-2 in pancreatic cancer correlates with decreased survival. *Gastroenterology* 1993, **105**, 1846–1856.
91. Sasada R, Ono Y, Taniyama Y, *et al*. Cloning and expression of cDNA encoding human betacellulin, a new member of the EGF family. *Biochem Biophys Res Commun* 1993, **190**, 1173–1179.
92. Derynck R. The physiology of transforming growth factor alpha. *Semin Cancer Biol* 1992, **58**, 27–52.
93. Davis-Fleischer KM, Besner GE. Structure and function of heparin-binding EGF-like growth factor (HB-EGF). *Frontiers Biosci* 1998, **3**, 288–299.
94. Carraway KL, Weber CK, Unger MJ, *et al*. Neuroregulin-2, a new ligand of ErbB3/ErbB4-receptor tyrosine kinases. *Nature* 1997, **387**, 512–516.
95. Laurence DJ, Gusterton BA. The epidermal growth factor: a review of structural and functional relationships in the normal organism and in cancer cells. *Tumour Biol* 1990, **11**, 229–261.
96. Dong M, Nio Y, Guo K-J, *et al*. Epidermal growth factor and its receptor as prognostic indicators in Chinese patients with pancreatic cancer. *Anticancer Res* 1998, **18**, 4613–4619.
97. Ito Y, Higashiyama S, Takeda T, *et al*. Expression of heparin-binding epidermal growth factor -like growth factor receptor in pancreatic adenocarcinoma. *Int J Pancreatol* 2001, **29**, 47–52.
98. Uegaki K, Nio Y, Inoue S, *et al*. Clinicopathological significance of epidermal growth factor and receptor in human pancreatic cancer. *Anticancer Res* 1997, **17**, 3841–3847.
99. Friess H, Yamanaka Y, Kobrin MS, *et al*. Enhanced erB-3 expression in human pancreatic cancer correlates with tumour progression. *Clin Cancer Res* 1995, **11**, 1413–1420.
100. Yamanaka Y. The immunohistochemical expressions of epidermal growth factor, epidermal growth factor receptors and c-erbB2 oncoprotein in human pancreatic cancer. *Nippon Ika Daigaku Zasshi* 1992, **59**, 51–61.
101. Yamanaka Y, Friess H, Kobrin MS, *et al*. Co-expression of epidermal growth factor receptor and ligands in human pancreatic cancer is associated with enhanced tumour aggressiveness. *Anticancer Res* 1993, **13**, 565–570.
102. Ueda M, Ogata S, Tsuda H, *et al*. The correlation between cytoplasmic overexpression of epidermal growth factor and tumour aggressiveness: poor prognosis in patients with pancreatic ductal adenocarcinoma. *Pancreas* 2004, **29**, 1–8.
103. Tamiolakis D, Venizelos I, Simopoulos C, *et al*. Correlation of immunohistochemically detected HER-2/neu (erbB-2) with histological stage and perineural invasion in pancreatic cancer. *Hepatogastroenterology* 2004, **51**, 334–337.
104. Tobita K, Kijima H, Dowaki S, *et al*. Epidermal growth factor expression in human pancreatic carcinoma: significance for liver metastasis. *Int J Mol Med* 2003, **11**, 305–309.
105. Zhang H, Yuan SZ. Expression of c-erbB2 oncogene protein, epidermal growth factor receptor and TGF-beta1 in human pancreatic ductal adenocarcinoma. *Hepatobiliary Pancreatic Dis Int* 2002, **1**, 620–623.
106. Koka V, Potti A, Koch M, *et al*. Role of immunohistochemical identification of Her-2/neu and detection of variability in

overexpression of pancreatic carcinoma. *Anticancer Res* 2002, **22**, 1593–1597.
107. Thybusch-Bernhardt A, Beckmann S, Juhl H. Comparative analysis of the EGF-receptor family in pancreatic cancer: expression of HER-4 correlates with a favourable tumour stage. *Int J Surg Invest* 2001, **2**, 393–400.
108. Safran H, Steinhoff M, Mangray S, *et al*. Overexpression of the HER-2/neu oncogene in pancreatic adenocarcinoma. *Am J Clin Oncol* 2001, **24**, 496–499.
109. Novotny J, Petruzelka L, Vedralova J, *et al*. Prognostic significance of c-erbB-2 gene expression in pancreatic cancer patients. *Neoplasma* 2001, **48**, 188–191.
110. Graber HU, Friess H, Kaufmann B, *et al*. ErbB-4 mRNA expression is decreased in non-metastatic pancreatic cancer. *Int J Cancer* 1999, **84**, 24–27.
111. Dugan MC, Dergham ST, Kucway R, *et al*. HER-2/neu expression in pancreatic adenocarcinoma: relationship to tumour differentiation and survival. *Pancreas* 1997, **14**, 229–236.
112. Okada N, Ohshio G, Yamaki K, *et al*. Elevated serum c-erbB-2 protein levels in patients with pancreatic cancer: correlation to metastasis and shorter survival. *Oncology* 1995, **52**(5), 392–396.
113. Lei S, Appert HE, Nakata B, *et al*. Overexpression of HER2/neu oncogene in pancreatic cancer correlates with shortened survival. *Int J Pancreatol* 1995, **17**, 15–21.
114. Kuniyasu H, Ellis LM, Evans DB, *et al*. Relative expression of E-cadherin and type IV collegenase genes predicts disease outcome in patients with resectable pancreatic carcinoma. *Clin Cancer Res* 1999, **5**, 25–33.
115. August P, Javerzat S, Bikfalvi A. Regulation of vascular development by fibroblast growth factors. *Cell Tissue Res* 2003, **8**, 204–210.
116. Montesano R, Vassalli JD, Baird A, *et al*. Basic fibroblast growth factor induces angiogenesis in vitro. *Acad Sci USA* 1986, **83**, 7297–7301.
117. Ohta T, Yammamoto H, Numata M, *et al*. Expression of basic fibroblast growth factor and its receptor in human pancreatic carcinomas. *Br J Cancer* 1995, **72**, 824–831.
118. Yamanaka Y, Friess H, Buchler M, *et al*. Overexpression of acidic and basic fibroblast growth factors in human pancreatic cancer correlates with advanced tumour stage. *Cancer Res* 1993, **53**, 5289–5296.
119. Jones L, Ghaneh P, Humphreys M, *et al*. The matrix metalloproteinases and their inhibitors in the treatment of pancreatic cancer. *Ann NY Acad Sci* 1999, **30**, 288–307.
120. Harvey SR, Hurd TC, Markus G, *et al*. Evaluation of urinary plasminogen activator, its receptor matrix metalloproteinase-9 and von Willebrand factor in pancreatic cancer. *Clin Cancer Res* 2003, **15**, 4935–4943.
121. Nakamura H, Horita S, Senmaru N, *et al*. Association of matrilysin expression with progression and poor prognosis in human pancreatic adenocarcinoma. *Oncol Rep* 2002, **9**, 751–755.
122. Yammamoto H, Itah F, Iku S, *et al*. Expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in human pancreatic adenocarcinomas: clinicopathologic and prognostic significance of matrilysin expression. *J Clin Oncol* 2001, **19**, 1118–1127.
123. Maata M, Soini Y, Liakka A, *et al*. Differential expression of matrix metalloproteinase (MMP)-2 and 9, and membrane type 1-MMP in hepatocellular and pancreatic adenocarcinoma: implications for tumour progression and clinical prognosis. *Clin Cancer Res* 2000, **6**, 2726–2734.
124. Gong YL, Xu GM, Huang WD, *et al*. Expression of matrix metalloproteinases and the tissue inhibitors of metalloproteinases and their local invasiveness and metastasis in Chinese human pancreatic cancer. *J Surg Oncol* 2000, **73**, 95–99.
125. Ito T, Ito M, Shiozawa J, *et al*. Expression of MMP-1 in human pancreatic carcinoma: relationship with prognostic factors. *Mod Pathol* 1999, **12**, 669–674.
126. Koshiba T, Hosotani R, Wada M, *et al*. Involvement of matrix metalloproteinase-2 activity in invasion and metastasis of pancreatic carcinoma. *Cancer* 1998, **82**, 642–650.
127. Cantero D, Friess H, Deflorin J, *et al*. Enhanced expression of urokinase plasminogen activator and its receptor in pancreatic cancer. *Br J Cancer* 1997, **75**, 388–395.
128. Joyce JA, Baruch A, Chehade K, *et al*. Cathepsin cysteine proteases are effectors of invasive growth and angiogenesis during multistage tumourigenesis. *Cancer Cell* 2004, **5**, 443–453.
129. Niedergethmann M, Hildenbrand R, Wolf G, *et al*. Angiogenesis and cathepsin expression are prognostic factors in pancreatic adenocarcinoma after curative resection. *Int J Pancreatol* 2000, **28**, 31–39.
130. Tumminello FM, Leto G, Pizzolanti G, *et al*. Cathepsin D, B and L circulating levels as prognostic markers of malignant progression. *Anticancer Res* 1996, **16**, 2315–2319.
131. Rohloff J, Zinke J, Schoppmeyer K, *et al*. Heparanase expression is a prognostic indicator for postoperative survival in pancreatic adenocarcinoma. *Br J Cancer* 2002, **22**, 1270–1275.
132. Kim EK, Xu S, Hollinger EF, *et al*. Human heparanase-1 gene expression in pancreatic cancer. *J Gastrointestinal Surg* 2002, **6**, 167–172.
133. Koliopanos A, Friess H, Kleeff J, *et al*. Heparanase expression in primary and metastatic pancreatic cancer. *Cancer Res* 2001, **61**, 4655–4659.
134. Takahashi K, Hasebe T, Oda T, *et al*. Cytoplasmic expression of laminin gamma2 chain correlates with postoperative hepatic metastases and poor prognosis in patients with pancreatic ductal adenocarcinoma. *Cancer* 2002, **94**, 1894–1901.
135. Gotoda T, Matsumara Y, Kondo H, *et al*. Expression of CD44 variants and its association with survival in pancreatic cancer. *Jap J Cancer Res* 1998, **89**, 1033–1040.
136. Gansuage F, Gansuage S, Bettina R, *et al*. Low serum levels of soluble CD44 variant6 are significantly associated with poor prognosis in patients with pancreatic carcinoma. *Cancer* 1997, **80**, 1733–1739.
137. Tomaszewska R, Nowak K, Stachura J. CD44 isoforms expression in intraductal and invasive pancreatic cancer and its correlation to p53 gene mutations. *Polish J Pathol* 1999, **50**, 145–153.
138. Bottger TC, Maschek H, Gottwald W, *et al*. Expression of CD44 and isoforms v4, v5, v6, v7, v10: new prognostic parameters in ductal pancreatic carcinoma. *Chirugie* 1998, **69**, 1089–1092.
139. Hosotani R, Kawaguchi M, Masui T, *et al*. Expression if integrin alphaVbeta3 in pancreatic carcinoma: link to MMP-2 activation and lymph node metastases. *Pancreas* 2002, **25**, 30–35.
140. Sawai H, Funahashi H, Matsuo Y, *et al*. Expression and prognostic roles of integrins and interleukin receptor type I in patients with ductal adenocarcinoma of the pancreas. *Digestive Dis Sci* 2003, **48**, 1241–1250.
141. Bottger TC, Maschek H, Lobo M, *et al*. Prognostic value of immunohistochemical expression of beta-integrin in pancreatic carcinoma. *Oncology* 1999, **56**, 308–313.
142. Doucas H, Garcea G, Manson M, *et al*. Changes in the Wnt signalling pathway in gastrointestinal cancers and their prognostic significance. *Eur J Cancer* 2005, **41**, 365–379.
143. Julkunen K, Makinen K, Karja V, *et al*. alpha, beta and chi-catenin expression in human pancreatic cancer. *Anticancer Res* 2003, **23**, 5043–5047.
144. Lowy AM, Fenoglio-Preiser C, Kim OJ, *et al*. Dysregulation of beta-catenin expression correlates with tumour differentiation in pancreatic duct adenocarcinoma. *Ann Surg Oncol* 2003, **10**, 284–290.

145. Joo YE, Rew JS, Park CS, *et al*. Expression of E-cadherin, alpha and beta-catenins in patients with pancreatic adenocarcinoma. *Pancreatology* 2002, **2**, 129–137.
146. Karayiannakis AJ, Syrigos KN, Polychroncdis A, *et al*. Expression patterns of alpha-, beta- and gamma-catenin in pancreatic cancer: correlation with E-cadherin expression, pathological features and prognosis. *Anticancer Res* 2001, **21**, 4127–4134.
147. Folkman J. How is blood vessel growth regulated in normal and neoplastic tissue? GHA. Clowes memorial Award Lecture. *Cancer Res* 1986, **46**, 467–473.
148. Folkman J. What is the evidence that tumours are angiogenesis dependent? *Natl Cancer Inst* 1990, **82**, 4–6.
149. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nature Med* 1995, **1**, 27–31.
150. Tischer E, Mitchell R, Hartman T, *et al*. The human gene for vascular endothelial growth factor. Multiple proteins are encoded through alternative exon splicing. *J Biol Chem* 1991, **266**, 11947–11954.
151. Kuwahara K, Sasaki T, Kuwada Y, *et al*. Expression of angiogenic factors in pancreatic ductal carcinoma: a correlative study with clinicopathological parameters and patient survival. *Pancreas* 2003, **26**, 344–349.
152. Karayiannakis AJ, Bolanaki H, Syrigos KN, *et al*. Serum vascular endothelial growth factor levels in pancreatic cancer patients correlate with advanced and metastatic disease and poor prognosis. *Cancer Letts* 2003, **194**, 119–124.
153. Buchler P, Reber HA, Buchler MW, *et al*. VEGF-RII influences the prognosis of pancreatic cancer. *Ann Surg* 2002, **236**, 738–749.
154. Niedergethmann M, Hildenbrand R, Wostbrock B, *et al*. High expression of vascular endothelial growth factor predicting early recurrence and poor prognosis after curative resection of ductal adenocarcinoma of the pancreas. *Pancreas* 2002, **25**, 122–129.
155. Fujioka S, Yoshida K, Yanagisawa S, *et al*. Angiogenesis in pancreatic carcinoma: thymidine phosphorylase expression in stroma cells and intratumoural microvessel density as independent predictors of overall and relapse-free survival. *Cancer* 2001, **92**, 1788–1797.
156. Seo Y, Baba H, Fukuda T, *et al*. High expression of vascular endothelial growth factor is associated with liver metastasis and poor prognosis for patients with ductal adenocarcinoma. *Cancer* 2000, **88**, 2239–2245.
157. Ikeda N, Adachi M, Taki T, *et al*. Prognostic significance of angiogenesis in human pancreatic cancer. *Br J Cancer* 1999, **79**, 1553–1563.
158. Fujimoto K, Hosotani R, Wada M, *et al*. Expression of two angiogenic factors, vascular endothelial cell growth factor and platelet derived endothelial cell growth factor in human pancreatic cancer, and its relationship to angiogenesis. *Eur J Cancer* 1998, **34**, 1439–1447.
159. Ellis LM, Takahashi K, Fenoglio CJ, *et al*. Vessel counts and vascular endothelial growth factor expression in pancreatic adenocarcinoma. *Eur J Cancer* 1998, **34**, 337–340.
160. Itakura J, Ishiwata T, Friess H, *et al*. Enhanced expression of vascular endothelial growth factor in pancreatic cancer correlates with local disease progression. *Clin Cancer Res* 1997, **8**, 1309–1316.
161. Minari Y, Nio Y, Hirahara N, *et al*. Heterogeneic distribution of thymidine phosphorylase between primary tumours and metastatic lesions of human pancreatic carcinoma. implications for the efficacy of chemotherapy with it or its derivatives. *Cancer Chemother Pharmacol* 2001, **47**, 415–422.
162. Takao S, Takebasyshai Y, Che X, *et al*. Expression of thymidine phosphorylase is associated with poor prognosis in patients with ductal adenocarcinoma of the pancreas. *Clin Cancer Res* 1998, **4**, 1619–1624.
163. Baenzinger NL, Brodie GN, Majerus PW. Isolation and properties of a thrombin-sensitive protein of human platelets. *J Biol Chem* 1972, **247**, 2723–2731.
164. Kasper U, Ebert M, Malfertheiner P, *et al*. Expression of thrombospondin-1 in pancreatic carcinoma: correlation with microvessel density. *Virchows Arch* 2001, **438**, 116–120.
165. Qian X, Rothman VL, Nicosia RF, *et al*. Expression of thrombosponind-1 in human pancreatic adenocarcinomas: Role of matrix metalloproteinase-9 production. *Pathol Oncol Res* 2001, **7**, 251–259.
166. Tobita K, Kijima H, Dowaki S, *et al*. Thrombospondin-1 expression as a prognostic predictor of pancreatic ductal carcinoma. *Int J Oncol* 2002, **21**, 1189–1195.
167. Shimoyama S, Gansuage F, Gansauge S, *et al*. Increased angiogenin expression in pancreatic cancer is related to cancer aggressiveness. *Cancer Res* 2003, **56**, 2703–2706.
168. Valerio A, Basso D, Mazza S, *et al*. Serum protein profiles of patients with pancreatic cancer and chronic pancreatitis: searching for a diagnostic protein pattern. *Rapid Commun Mass Spectrom* 2001, **15**, 2420–2425.
169. Rosty C, Goggins M. Identification of differentially expressed proteins in pancreatic cancer using a global proteomic approach. *Methods Mol Med* 2003, **103**, 189–198.
170. Battacharyya S, Siegel ER, Peterson GM, *et al*. Diagnosis of pancreatic cancer using serum proteomic profiling. *Neoplasia* 2004, **5**, 674–686.