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REVIEW TOPIC: PANCREATIC CANCER

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Molecular pattern of ductal pancreatic cancer

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Abstract Our understanding of the molecular pathology underlying the development and progression of ductal pancreatic cancer has been revolutionised during the last 5 years due to the spectacular development of novel molecular biological techniques. In the present article, we describe key molecular alterations of sporadic and inherited ductal pancreatic cancer. Overexpression of growth factors and growth factor receptors are present in a significant proportion of this tumour type. Mutation of the K-ras oncogene, and disruption of p53 or p16 tumour suppressor gene abrogates the control of the cyclin-dependent kinases (cdk) and retinoblastoma (Rb) gene pathway, causing continuous growth of the pancreatic tumour. Inactivation of the SMAD4 tumour suppressor gene leads to loss of the inhibitory influence of the transforming growth factor β signalling pathway. Lost or decreased expression of retinoid receptors and failure of telomerase activity may play a role in pancreatic carcinogenesis. Tumour-associated proteinases, matrix metalloproteinases and plasminogen activators are reported to be involved in pancreatic cancer invasion and metastasis. Furthermore, the cytogenetic changes in this cancer are summarised. This molecular pattern distinguishes pancreatic cancer from other epithelial tumours and represents a promising basis for the development of diagnostic and other clinical applications.

Key words Pancreatic cancer · Oncogene · Tumour suppressor gene · Growth factors · Growth factor receptors

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Introduction

As a result of the application of modern molecular biological techniques to cell systems in vitro and human tumours in vivo, there is increasing information about the genetic basis of cancer. The development and growth of pancreatic adenocarcinoma involves oncogene activation, loss of tumour suppressor gene function and overexpression of receptor-ligand systems (Fig. 1.). This article reviews the current knowledge of the molecular and cellular pattern of pancreatic cancer which will lead to novel approaches to early diagnosis and the development of new therapeutic and follow-up stratagems.

Oncogenes and growth factor systems implicated

K-ras

K-ras is one of the three members of the human RAS gene family that code for highly related 21-kDa proteins with guanosine triphosphatase (GTPase) activity. It was first identified as the transforming principle of the Kirsten strains of rat sarcoma virus and has been assigned to the short arm of chromosome 12 (12p12.1-pter). RAS proteins, which are synthesised in the cytosol and attached to the inner side of the plasma membrane after post-translational modifications, have functional and structural resemblance to the regulatory (G) proteins. It has now been shown that they play a role in transduction of signals from the cell surface.

Naturally occurring mutations in the K-ras oncogene have been localised to codons 12, 13 and 61. The presence of a glycine residue at codon 12 appears to be necessary for the normal function of RAS proteins. Substitution of glycine by any other amino acid residue (with the exception of proline) results in the oncogenic activation of these molecules. The mutants result in a reduced rate of guanosine triphosphate (GTP) hydrolysis and a resistance to the

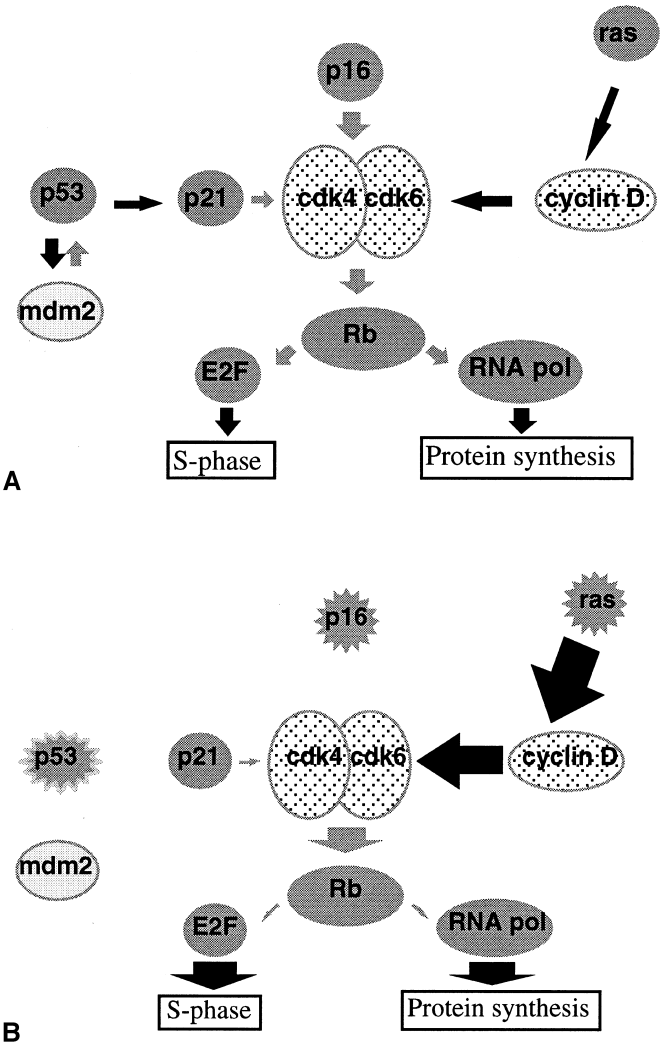

Fig. 1 Cell-cycle regulatory pathway in normal cell (**A**) and in the development of pancreatic cancer cell (**B**). Disruption of *p53* or *p16*, and maturation of the K-ras oncogene abrogates the control of the cyclin-dependent kinases (*cdk*) and retinoblastoma (*Rb*) gene pathway, causing continuous growth of the pancreatic tumour (*black arrows*, upregulation; *grey arrows*, downregulation)

inhibitory effects of GTPase-activating proteins (GAPs). The mutant RAS proteins are therefore locked into an active GTP-bound state and transmit a constitutive growth signal to the nucleus.

The incidence of mutated K-ras genes varies widely among different tumour types. The highest incidence is found in pancreatic carcinomas and, since 1988, more than 50 reports have described K-ras mutation in this tumour type. However, the reported prevalence of activating mutations varies from 47% to 100% [1, 2, 3, 4, 5]. RAS mutations are also found frequently in colorectal carcinomas, adenocarcinomas of lung and follicular and undifferentiated thyroid carcinomas, whereas in several other tumour types a mutated gene is only found occasionally [6].

The K-ras mutations present in pancreatic carcinomas are almost exclusively at codon 12, and analysis of the specific nucleotide reveals a heterogeneous type of mutation [3, 4, 7, 8]. This might be caused by multiple carcinogens and/or diverse errors during DNA replication and repair rather than a single, specific mutagen. The most common mutation at codon 12 is the second base change from guanine (G) to adenine (A) transition (GGT to GAT), similar to those in colorectal tumours, while guanine to thymine (G to T) transversion (GGT to GTT) is more prevalent in lung carcinomas. In contrast, the aspartic acid mutation at codon 13 (GGC to GAC) is relatively frequent in colorectal tumours, but rare in pancreatic and lung carcinomas [7].

The differences in the mutation spectrum of the K-ras gene in cancers of the gastrointestinal and respiratory tracts are suggestive of differential exposure to genotoxic agents. The mutation in pancreatic cancer may be produced by a different type of carcinogen, such as aromatic amines and *N*-nitrosamines, while benzpyrene, a tobacco carcinogen, is thought to be the cause of lung cancers [9]. Moreover, a recent study showed the prevalence of K-ras mutation in cases associated with alcohol consumption (one of the risk factors for pancreatic carcinoma) to be three times higher than in non-drinkers [10]. In addition, the mutational pattern in cancers among the different European countries, and when compared with Japanese, shows remarkable differences, both in the site of the mutation (first or second base) and in the ratio of transitions over transversions.

K-ras mutations are also found in other pancreatic tumours of ductal origin, such as cystadenoma, cystadenocarcinoma [11] and intraductal papillary mucinous tumours (IPMT) [12, 13]. Recently, one study showed that a stepwise increase in the frequency of K-ras mutations at codon 12 correlated with the stage of neoplastic evolution to cancer [14]. The mutations are observed as frequently in patients with IPMT (81%) as in patients with pancreatic carcinomas. They found a relatively high number of guanine to cytosine (G to C) transversions in IPMT, different from the guanine to adenine (G to A), the most common base change in pancreatic cancer. While all endocrine tumours of the pancreas were thought to be negative for mutations at codon 12 of K-ras gene, a recent study showed that four of six patients with malignant insulinoma and two of six patients with benign insulinoma harboured K-ras point mutations at codon 12. All patients with a mutated K-ras oncogene also had elevated levels of p53 protein, c-myc and Transforming Growth Factor-alpha (TGFα) [15].

Since the majority of pancreatic carcinomas have a mutated K-ras gene, the presence of the mutation has been proposed as a molecular genetic marker for malignancy. Several studies [16, 17, 18, 19] reported that detection of K-ras mutations in fine needle aspirates of pancreatic lesions increases the sensitivity of diagnosis for this cancer. The detection of a K-ras mutation in pancreatic juice, bile, peripheral blood and also in stool samples has been reported [20, 21, 22, 23, 24]. However, K-ras mutations are not only found in pancreatic carcinomas, but also in mucinous hypertrophy and ductal papillary hyperplasia [5, 24, 25, 26]. Recent studies also found a high frequency of K-ras mutations in hyperplastic ducts associated with chronic pancreatitis [27, 28]. This provides a genetic basis for the potential progression of chronic pancreatitis to pancreatic

carcinoma and suggests that K-ras mutations are an early event in pancreatic carcinogenesis. Although the risk of pancreatic cancer was significantly increased in patients with chronic pancreatitis with the cumulative risk of 1.8% and 4% at 10 years and 20 years after diagnosis of pancreatitis [29], a recent study reported that the finding of K-ras mutations in patients with chronic pancreatitis had no direct clinical relevance to the development of pancreatic cancer during a follow-up 140 months [30].

It appears that in the absence of a co-operating genetic event, such as inactivation of the tumour suppressor gene p16, the lesions containing cells with K-ras mutations are self-limiting (by apoptosis after a strictly limited number of cell divisions, as shown by transfection experiments [31]) and the risk of progression to full malignancy is probably around 1% or less. Likewise, a recent study suggested that inactivating mutations of the p16 gene following the K-ras mutation extend the progression of pancreatic carcinoma [32]. The presence of K-ras mutations does not appear to be a useful prognostic marker in pancreatic carcinoma. While the survival rate of lung cancers correlated positively with the absence of K-ras mutations [33, 34], and G to T and G to C transversions conferred a poorer prognosis than G to A transitions in colonic carcinomas [35, 36], no correlations were found between K-ras genotype and tumour size, histological grade, tumour ploidy, tumour proliferation index or patient survival of pancreatic carcinomas [3, 26, 37, 38, 39].

Epidermal growth factor receptor family

Epidermal growth factor (EGF) receptor (EGFR) (also known as c-erbB) is a transmembrane protein with intrinsic tyrosine kinase activity which is involved in normal cellular growth and differentiation [40]. The EGFR gene is usually overexpressed at high levels in pancreatic adenocarcinoma due to increased gene transcription [41, 42]. To date, at least 15 EGF-like ligands have been described, including TGFα, amphiregulin (AR), heparin-binding EGF-like growth factor (HB-EGF), betacellulin and epiregulin. These ligands form a complex series of interactions with different members of the EGF receptor family that promote either homo- or hetero-dimerization [43, 44]. EGF is a potent mitogenic stimulus for cells in different tissues, including pancreatic cells [45].

A recent report shows that EGF evoked a strong proliferative response in all pancreatic cell types studied [46]. Pancreatic carcinomas overexpress EGF and TGFα (12–46% and 50–95% of cases, respectively) compared with normal human pancreatic tissue [47, 48]. It was shown that human pancreatic cancers exhibit increased EGF and TGFα immunostaining [48]. Furthermore, co-expression of the receptor and both EGF and TGFα ligands is found in 20% of pancreatic tumours, and co-expression of the receptor and one of the two ligands in 18% more [47].

In the normal pancreas, AR appears to be in the nuclei of ductal cells, while it is also present in the cytoplasm of the ductal adenocarcinoma cells [49]. There is a significant correlation among cytoplasmic AR immunoreactivity in pancreatic cancer tissues, advanced tumour stage and shorter post-operative survival [50]. In a recent study, AR was shown to have the potential to act as an autocrine growth factor in pancreatic cancer cells [51]. In vitro, HB-EGF enhanced the growth of human pancreatic cancer cell lines in a dose-dependent manner, with a potency that was generally similar to that of EGF and TGFα. Immunohistochemical analysis revealed the presence of HB-EGF immunoreactivity in pancreatic cancer cells in 50% of the tumours [52].

Epiregulin is a novel EGF family member that exhibits bifunctional regulatory properties as it stimulates the growth of many types of cells including fibroblasts and hepatocytes, while inhibiting the growth of several epithelial tumour cells [53]. The concomitant overexpression of the EGFR and one or more of its ligands appears to be a marker of poor prognosis and has been correlated with enhanced ability of certain tumours to invade normal tissue and metastasise, and a shorter postoperative survival period [42, 47, 54].

EGFR belongs to a family of closely related transmembrane proteins that include HER-2/neu (also known as c-erbB-2), HER3 (c-erbB-3) and HER4 (c-erbB-4) [55, 56]. HER-2/neu appears to bind a family of ligands, whereas HER3 and HER4 bind to the family of heregulins. In addition, HER4 also binds to betacellulin, a potent mitogen produced by the β cells of the islets of Langerhans [57]. It has been shown that HER-2/neu and HER3 are frequently overexpressed in pancreatic cancers [58, 59]. Amplification of HER-2/neu has been found in a significant number of cell lines of other origin [60].

There are various data concerning the incidence of HER-2/neu expression in invasive ductal adenocarcinomas [58, 61, 62, 63] and in intraductal mucin-hypersecreting neoplasms of the pancreas [63]. A recent study showed that the pattern of HER-2/neu expression was related to glandular differentiation and early oncogenesis of pancreatic cancer, but not related to survival differences [64]. In contrast, HER3 was found to be associated with advanced tumour stage and shorter postoperative survival [65].

Hepatocyte growth factor and the met receptor

Hepatocyte growth factor (HGF), otherwise known as scatter factor (SF), is a ligand for the c-met receptor tyrosine kinase. It is a mesenchymal- or stromal-derived multipotent polypeptide which mediates epithelial-mesenchymal interactions [66]. HGF has been implicated in the regulation of mitogenesis, motogenesis and morphogenesis [67]. In vivo, it plays a role in tissue regeneration, tumour progression and embryological processes, which generally require both cell motility and cell proliferation. Over the past few years, the structure, function and signal transduction pathways of HGF and its receptor have become

clearer. It is now known to play an important part in the regulation of both normal physiological and pathological processes [68].

HGF is detectable at low levels in the normal human exocrine pancreas, but it is upregulated 10-fold in the majority of pancreatic ductal adenocarcinomas [69, 70]. The MET receptor has also been observed to demonstrate weak expression in the normal pancreas, but markedly increased expression in pancreatic carcinoma [71, 72]. This supports the assumption that HGF could act as a growth-promoting factor on this cancer. In vitro, phosphorylation of the MET receptor in human pancreatic cell lines by HGF stimulates their movements. If there is overexpression of the MET receptor, constitutive autophosphorylation can occur even in the absence of exogenous ligand. It can be concluded that upregulation of this system may stimulate growth and enhance motility and, therefore, may be an important step in the progression of metastatic spread [69, 71].

One study has shown that HGF and MET expression may play significant bifunctional roles during carcinogenesis and progression of human pancreatic ductal carcinoma. An upregulation of MET expression and HGF–MET interaction may have an important pathogenic role during the early stages of neoplastic promotion, whereas modulation of MET expression in invasive pancreatic adenocarcinoma appears to result in a more aggressive clinical behaviour [73].

Transforming growth factor beta family

The transforming growth factor beta (TGFβ) superfamily consists of large numbers of regulatory polypeptides which include several TGFβ isoforms as well as activin, inhibin, bone morphogenetic proteins (BMPs), Mullerian-inhibiting substance (MIS) and growth and differentiation factors (GDFs) [74, 75]. Three isoforms of TGFβ (TGFβ_1, TGFβ_2 and TGFβ_3) exist in mammals. TGFβs possess three major activities: (1) they inhibit proliferation of most cells, but can stimulate the growth of some mesenchymal cells; (2) they exert immunosuppressive effects; and (3) they enhance extracellular matrix formation [76]. All three TGFβ isoforms are expressed in both the exocrine and endocrine constituents of the normal pancreas. TGFβ_1 protein expression was present in a large number of acinar cells, whereas TGFβ_2 and TGFβ_3 immunoreactivity was more intense in islets; all three isoforms have the same intensity of immunostaining in ductal cells [77].

In human pancreatic cancers, there are increased levels of TGFβ isoforms and enhanced TGFβ mRNA expression [78, 79]. Only the expression of the TGFβ_2 isoform correlated with advanced tumour stage, indicating that, in pancreatic cancer, the presence of the TGFβ_2 isoform may be a marker for progression [77]. Nevertheless, increased TGFβ expression may also occur in degenerating non-neoplastic cells next to the invasive tumour. This suggests that the upregulation of the TGFβ isoforms should not be considered to be tumour-specific [80].

There are three major receptors: TGFβRI, TGFβRII and TGFβRIII. Two types of the receptors (type I and type II) possessing a serine/threonine kinase activity within their cytoplasmic domains are involved in signal transduction [81]. Overexpression of TGFβRII may result from either increased gene transcription rate or prolonged TGFβRII mRNA stability in pancreatic cancer cells in vivo, whereas TGFβRIII mRNA levels are not increased. Thus, enhanced levels of TGFβRII may have a role in regulating human pancreatic cancer cell growth, while TGFβRIII may function in the extracellular matrix [80].

Fibroblast growth factors and receptors

The fibroblast growth factors (FGF) are a family of structurally related polypeptides that are mitogenic for a broad range of cell type as well as mediators of a wide spectrum of developmental and pathophysiological processes, both in vivo and in vitro [82]. The FGF family presently comprises 10 members (FGF1–10). The FGF1 (acidic) and FGF2 (basic) are closely related prototypes of this family; each is chemotactic toward fibroblasts, participates in tissue repair and promotes angiogenesis [83]. They are detected at low levels in the normal pancreas and exhibit increased expression at the mRNA levels in chronic pancreatitis [84, 85]. There is increased expression of FGF1 and FGF2 in pancreatic carcinomas (60% and 50% of cases, respectively). These appears to be a significant correlation between the expression of either FGF1 or FGF2 in the cancer cells and advanced tumour stage [86].

FGFs stimulate cellular response by binding to and activating a family of transmembrane-receptor tyrosine kinases. There are four distinct high-affinity FGF receptors (FGFR1–4). FGFR1 and FGFR2 are expressed in a wide variety of cell types, while FGFR3 and FGFR4 have a more restricted pattern of expression [87]. In the normal pancreas, all FGFRs are found at relatively low levels [84], whereas significant expression of FGFR1, 3 and 4 is found in approximately 60% of pancreatic cancers [84, 88, 89]. A recent study also found that the expression of FGF2 in human pancreatic carcinoma is strongly associated with the proliferation of tumour cells and intra-tumour endothelial cells and its increased expression may give tumour cells a growth advantage [90].

Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF), is an endothelial cell-specific mitogen and a regulator of physiological and pathological angiogenesis. The VEGF family comprises secreted homodimeric proteins encoded by a single gene. Alternative splicing of VEGF pre-mRNA leads to four isoforms having 121,165, 189 and 206 amino acid residues in the mature monomer [91, 92]. All four forms are mitogenic

to vascular endothelial cells and induce vascular permeabilization. VEGF signals through two high-affinity transmembrane receptors which are class-III transmembrane protein tyrosine kinases. VEGF receptor-1 was originally named the fms-like tyrosine kinase, and is also known as flt-1. VEGF receptor-2, also known as KDR, is the human homologue of flk-1 [93].

VEGF expression has been demonstrated in a number of human cancer cell lines suggesting a trophic role for VEGF in supporting tumour growth via host angiogenesis [94]. In normal pancreatic tissue, VEGF immunoreactivity was found in islet cells, which are known to express and secrete VEGF [95]. In duct ligation-induced acute pancreatitis, numerous inflammatory leucocytes containing VEGF were seen to infiltrate between hyperplastic ducts [96]. This indicates the possibility that VEGF plays a role in the paracrine regulation of ductal growth and differentiation. A recent study showed a 5.2-fold increase in VEGF mRNA transcript in pancreatic cancer samples when compared with normal pancreatic tissues. This finding was associated with increased blood-vessel number, larger tumour size and enhanced local spread, but not with decreased patient survival [97]. Hence this factor may contribute to the angiogenic process and tumour growth in this disease.

Retinoids and receptors

Retinoids are natural and synthetic derivatives of vitamin A [98]. They elicit a large array of biological responses during morphogenesis and differentiation [99]. Similar to the steroid hormone superfamily, the pleiotrophic biological effects of retinoids are mediated by two families of nuclear retinoic acid (RA) receptor: the RA receptors and the retinoid X receptors, each consisting of three receptors subtypes of α, β and γ. Both receptors act as ligand-activated transcription factors, controlling gene transcription initiated from promoters of retinoid-regulated genes by interacting with *cis*-acting DNA elements, the so-called RA responsive elements [100, 101].

There has been increasing evidence that retinoic acid and vitamin D are naturally occurring agents controlling cellular differentiation and proliferation both in normal and malignant cells [102]. In vitro, it has been shown that vitamin D analogues, together with retinoids, inhibit the growth of human pancreatic cancer cells [103]. In addition, a recent study found that retinoids decrease pancreatic carcinoma cell adhesion to laminin, one of the major constituents of basement membranes, which results in growth inhibition [104]. RA receptor β expression (RARβ) is known to be lost or decreased during malignant transformation in human pancreatic adenocarcinoma [105]. One study suggested that overexpression of RARβ in the human pancreatic carcinoma cell line inhibited cellular proliferation in vitro and in vivo [106]. Exploitation of RAR expression may provide rational strategies for retinoid treatment of pancreatic cancer in the future.

AKT2

AKT2 was identified as one of the human homologues of the *v-akt* oncogene [107]. Human AKT2 is located at 19q13.1-q13.2 and encodes a protein-serine/threonine kinase which has significant sequence and structural homology to members of the protein kinase C and cyclic adenosine monophosphate-dependent protein kinase families [107]. It is activated by platelet-derived growth factor (PDGF), EGF, basic FGF and insulin through phosphatidylinositol-3-OH kinase, suggesting that it is an important signal mediator that may contribute to the control of cell proliferation and malignant transformation [108]. AKT2 has been shown to be amplified or overexpressed in approximately 10% of primary tumours and cell lines of pancreatic cancer [109]. Interestingly, this study also showed the reduction of tumour growth and invasiveness in pancreatic tumour cell lines transfected with anti-sense AKT2 RNA. Such results suggest that anti-sense inhibition of AKT2 may be a potentially important approach for pancreatic cancer gene therapy.

Tumour suppressor genes

The p53 gene

The p53 gene is located on the short arm of chromosome 17 (17p13) and encodes a 53-kDa nuclear phosphoprotein (p53 protein) which functions as a transcription factor. In response to DNA damage, p53 appears to direct the transcription of the p21 WAF1 gene, which is an inhibitor of cyclin-dependent kinases. This suggests that p53-dependent p21 induction is responsible for arresting the cell cycle in G1, following DNA damage. Moreover, p53 protein is also required to direct cells into apoptosis when the level of DNA damage is too great.

Inactivation of the p53 gene is the most common genetic alteration found in human malignancies, and mutations and/or deletions occur very frequently in pancreatic carcinomas (40–70% of cases) [110, 111, 112, 113, 114]. Transitions represent the majority of the point mutations (70%) in pancreatic cancers, while transversions (which have been seen at high frequency in smoking-related carcinomas, such as lung, oesophageal and head and neck tumours) were not common. Most mutations in pancreatic carcinomas affect G:C sites with a predominance of transitions (70%), mainly represented by G:C to A:T substitutions, reminiscent of the pattern observed in colonic carcinomas (75% of transitions) [112]. Unlike other cancers, such as liver and colonic cancers, no specific site seems to be preferentially affected in p53 mutations of pancreatic cancers. The missense mutations are scattered through numerous codons, such as at 135, 158–160, 248, 273, 282 and 286, but are mainly confined to four regions of the gene that are conserved through evolution, located in exons 5–8. There is a peculiar preference for pancreatic carcinomas to have intragenic deletions and, in particular, 1–2 bp micro-

deletions. These deletions frequently were within repeated runs of single nucleotides or nucleotide pairs.

Due to an increased half-life and accumulation of p53 protein caused by p53 mutation, one study recently reported that p53 protein concentrations in serum of pancreatic cancer patients were significantly higher than in chronic pancreatitis patients or healthy persons. The concentrations were also significantly higher in those patients presenting with tumours with distant metastases than in those without [115], results similar to findings in colonic and lung cancers [116, 117]. Thus, serum p53 protein concentrations may be an additional tumour marker in pancreatic cancer patients.

The p16 gene (MTS1/CDKN2/CDKN41/p16^{INK4A}/DPC3)

p16 is one of the inhibitors for cyclin-dependent kinases (CDKs) which complexes with cyclin D1 and drives a cell's progression through the division cycle. p16 acts on RB1 to form a negative feedback loop that regulates the ability of RB1 to prevent cell proliferation. Deletions or mutations in the p16 gene may affect the relative balance of functional p16 and cyclin D, resulting in abnormal cell growth. It consists of three exons which encode a 148-amino acid protein of M_r 16 kDa [118]. Mutations of the p16 gene occur in pancreatic cancer at a rate of 30–82%, higher than that reported in any other tumour type [119, 120]. Although the RB1, p16, cyclin D1 and CDK4 pathway in other tumour types can be abrogated by inactivation of any of the members of the pathway, in pancreatic carcinoma, this pathway appears to be almost exclusively inactivated by alterations of the p16 protein [121]. Thus, p16 inactivations involve a strong tissue-specificity. p16 is located at chromosome 9p21, a region that is linked to familial melanoma and is homozygously deleted in many tumour cell lines [122, 123, 124]. Of interest, this gene may play a major role as a tumour suppressor gene in a subset of the familial atypical mole-multiple melanoma (FAMMM) syndrome with pancreatic cancer.

The p16 alterations in pancreatic carcinomas may be caused by a number of mechanisms, including hetero- or homo-zygous deletions, coding-sequence mutations and transcriptional silencing. Loss of function usually occurs by loss of heterozygosity (90%) or by homozygous deletion (40%) which frequently extends to involve another candidate suppressor gene p15 (MTS2) at a closely adjacent locus. Homozygous co-deletion of p16 and p15 has been reported at the rate of 46% in pancreatic cancers [125]. p15 also acts as an effector of TGFβ-induced cell-cycle arrest and may be important in pancreatic carcinogenesis, though there is no direct evidence of p15 being a tumour suppressor gene.

Gene deleted in pancreatic carcinoma, locus 4 (DPC4) (SMAD4)

In one small area (about 1% of the length of the chromosome) of chromosome 18q, 48% of pancreatic carcinomas [126] were found to have homozygous deletions at the site 18q21.1 [127]. Because this was the fourth locus to be investigated for this special form of deletion in pancreatic cancer, the cloned gene was initially named DPC4. The coding sequence of this gene is 1660 nucleotides in length, covering 11 exons. It is very similar (75–85% at the sequence level) to a Drosophila gene, *mothers against dpp* (MAD) which is needed in early fly development; thus, DPC4 has been renamed SMAD4. The signals are part of the TGFβ superfamily of signaling pathway, which usually act to promote differentiation and to slow the growth of cells. As in pancreatic carcinoma, a recent study in a breast cancer cell line also confirm that SMAD4 is part of the TGFβ signal transduction pathway, leading to growth inhibition and plasminogen activator inhibitor 1 (PAI-1) induction [128]. Moreover, it has been shown that this gene contains a transcriptional activation domain [129]. Mutations at this gene are also frequently detected, including nonsense mutations in exons 8, 9 and 11, missense mutations at exon 11, and splice donor-site mutation after exon 10 [127]. In a recent study, no DPC4 mutations were found in 25 individuals (8 with pancreatic carcinoma) from 11 kindreds with a familial history of pancreatic carcinoma. Thus, DPC4 does not appear to be a candidate for the gene responsible for the familial form of pancreatic carcinoma [130].

Other tumour suppressor genes

Retinoblastoma (RB1) gene is located at 13q14 and is an important tumour suppressor gene, found to be mutated in numerous tumour types. However, inactivation of RB1 rarely occurs in pancreatic carcinoma. A study showed the evidence of RB1 allele loss in only 2 of 32 (6%) pancreatic cancers, and no evidence for loss of the gene deleted in colorectal carcinoma (DCC) was found [131]. Thus, the alterations of RB1 and DCC are unlikely to play a major role in pancreatic carcinogenesis. There have been reports of a high prevalence of allelic imbalance and loci noted to be homozygously deleted in pancreatic cancer, which have been labelled DPC loci 1 and 2. These co-localise at the 13q12 region of the breast cancer gene, BRCA2. Suspicions are therefore raised that the BRCA2 gene might represent the pancreatic cancer tumour suppressor gene on 13q, and there is evidence that breast cancers and pancreatic cancers can have a shared pattern of inheritance.

Telomerase

Telomerase is an enzyme that contains an RNA template complementary to GGTTAG repeats and is believed to be involved in the de-novo synthesis of GGTTAG telomeric DNA onto chromosomal ends. Almost all normal somatic cells have no detectable telomerase activity, except for the proliferative cells of self-renewal tissues such as haematopoietic progenitor cells, lymphocytes, skin basal cells and

intestinal stem cells. Activation of telomerase and stabilisation of telomerase are considered to be necessary for immortalisation of human tumour cells. Telomerase activity is (at higher frequency) detected in a wide range of cancers, such as neuroblastomas (94%), lung cancers (80%), colorectal cancers (93%), hepatocellular carcinoma (85%), gastric cancers (85%) and breast cancers (93%). In pancreatic carcinoma, it was detected in 95–100% of cases, but was detectable in none of the benign pancreatic tumours examined [132, 133]. This suggests that telomerase activity may play a role in pancreatic carcinogenesis. Moreover, eight ex vivo brushing samples of the duct of pancreatic cancers demonstrated telomerase activity, whereas no detectable activity in four samples of benign lesions [132]. Thus, telomerase activity may be a specific marker, and the detection in brushing samples or pancreatic juice may be useful in the early diagnosis of this tumour.

Tumour-associated proteinases

Evidence has accumulated that different types of tumour-associated proteinases, their inhibitors and their receptors are involved in tumour invasion and metastasis. The degradation of extracellular matrix (ECM) components is an important phase, and many secreting enzymes, matrix metalloproteinases (MMPs) and plasminogen activators (PAs) may play a major role in cancer invasion and metastasis.

Matrix metalloproteinases

MMPs are a gene family of zinc-dependent endopeptidase enzymes with a broad spectrum of proteolytic activity for most components of the ECM. They are composed of at least thirteen different gene products and subdivided into four groups, depending on their substrate specificity and structural similarity: the specific collagenases, the gelatinases, the stromelysins and a putative MMP [134]. The activities of MMPs in vivo are strictly regulated by tissue inhibitors of metalloproteinases (TIMPs) which include TIMP-1, TIMP-2 and TIMP-3.

Pancreatic cancer cell lines have been shown to exhibit collagenolytic and gelatinolytic activity due to MMP activity [135, 136]. Bramhall et al. have shown that immunoreactivities for MMP-2 (72-kDa collagenase IV), MMP-3 (stromelysin-1) and TIMP-1 were greatest in adenocarcinomas of the pancreas and ampulla compared with those in normal tissues or other pathologies. The results also implicate MMP-2, MMP-3 and TIMP-1 in the invasive phenotype of pancreatic and ampullary carcinoma [137]. The aggressive phenotype of pancreatic carcinoma may occur because of overexpression of MMP-2 and the reduction of expression of TIMP-2 [138]. Although it remains unclear which MMPs or TIMPs are associated with metastatic potentials of human pancreatic cancer, a recent in vivo study indicated that MMP-1 and MMP-2 might be positively associated with liver metastasis of this tumour [139]. Interestingly, Zervos et al. recently reported that the MMP inhibitor, BB-94 (Batimastat), limited the proliferation rate of pancreatic cell line in a dose-dependent fashion without direct cytotoxic effect in vitro [140]. Furthermore, pancreatic tumours in animals treated with BB-94 were significantly reduced in weight, volume and metastatic potential, which corresponded to increased animal weight and prolonged survival.

Urokinase-type plasminogen activator (uPA)

The conversion of plasminogen to active plasmin is thought to be a crucial step in the process of extracellular matrix degradation associated with metastatic spread. Activation of plasminogen is initiated by urokinase plasminogen activator (uPA) and tissue plasminogen activator (tPA). Plasmin degrades fibrin and a number of other components of the extracellular matrix, such as type-IV collagen, fibronectin and laminin, and also activates latent collagenase to potentiate their lytic activity. This promotes the passage of tumour cells through tissue barriers.

uPA is one of the components of the fibrinolytic cascade and acts as a broad-spectrum proteolytic enzyme involved in different physio-pathological processes, including cellular fibrinolysis, adhesion, migration, invasion and remodelling. The high affinity binding of uPA to the cell surface receptor (uPAR) accelerates plasmin generation from plasminogen and localises uPA activity to the cell surface. Expression of uPA has been reported in cancer of the breast, ovary, stomach, oesophagus, colon, lung and kidney. By immunohistochemical study, uPA expression was demonstrated in 76 of 97 (78%) pancreatic carcinoma specimens [141]. Interestingly, a recent study showed a sixfold and a fourfold increase in uPA and uPAR mRNA expression in pancreatic cancer, respectively, compared with normal samples. In addition, patients with the concomitant overexpression of uPA and uPAR had a shorter postoperative survival than patients in whom only one or none of these factors was overexpressed [142].

Cytogenetics

The somatic genetic changes which characterise pancreatic adenocarcinoma are still rather unclear. The identification of acquired genomic alterations would lead to further understanding of the biology of this neoplasm.

The largest number (62 cases) of pancreatic cancers have been studied by cytogeneticists at The Johns-Hopkins Hospital in an attempt to identify important chromosome abnormalities [143]. The most frequent findings are whole-chromosomal gains if chromosomes 7 and 20, and whole-chromosomal losses of chromosomes 6, 12, 13, 17 and 18. Structural abnormalities are common: the chromosomal arms most frequently involved are 1p, 1q, 3p, 6q, 7q, 11p, 17p and 19q. Portions of the long arm of chromosome 6 appear to be lost in approximately 8% of cases. Of partic-

ular interest, double minute chromosomes (DMs) are found in approximately 8% of tumours; they are observed in 31% of tumours with abnormal chromosomes. DMs are one of the two cytogenetic manifestations of gene amplification.

A recent review of the structural basis of molecular genetic alterations indicates that approximately two-thirds of chromosomal arms with allelic losses in pancreatic carcinomas have corresponding chromosomal structural abnormalities [144]. Furthermore, it also confirms that homozygous deletions are often small and beyond the limits of detection of classical cytogenetics. There are some of the apparent discrepancies between molecular and karyotypic analyses which can be explained by chromosomal or subchromosomal loss with reduplication of the remaining chromosome.

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