

Review

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Genetic alterations in pancreatic carcinoma

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Abstract

Cancer of the exocrine pancreas represents the fifth leading cause of cancer death in the Western population with an average survival after diagnosis of 3 to 6 months and a five-year survival rate under 5%. Our understanding of the molecular carcinogenesis has improved in the last few years due to the development of novel molecular biological techniques. Pancreatic cancer is a multi-stage process resulting from the accumulation of genetic changes in the somatic DNA of normal cells. In this article we describe major genetic alterations of pancreatic cancer, mutations in the proto-oncogene *K-RAS* and the tumor suppressors *INK4A*, *TP53* and *DPC4/SMAD4*. The accumulation of these genetic changes leads to a profound disturbance in cell cycle regulation and continuous growth. The knowledge of the underlying molecular mechanisms will offer new therapeutic and diagnostic options and hopefully improve the outcome of this aggressive disease.

Review

Cancer of the exocrine pancreas represents the fifth leading cause of cancer death in the Western population with a five-year survival rate under 5% [1]. Because of the few treatment options, understanding of the molecular pathology is prerequisite to identify potential molecular targets for drug therapy.

The PanIN (Pancreatic intraepithelial neoplasia) classification describes various changes in the pancreatic duct system distinguishing three PanIN grades (PanIN 1 – PanIN 3) according to the degree of structural dysplasia and cytological atypia present in the lesions [2]. Microdissection techniques revealed genetic alteration in cancer-causing genes in the putative premalignant lesions similar to pancreatic carcinomas (see table 1). The combination of morphological and genetic observations leads to a tumor progression model for pancreatic carcinoma, comparable to the adenoma-carcinoma sequence in colorectal carcinomas [3]. The sequential acquisition of mutations in the proto-oncogene *K-RAS* and the tumor suppressors

INK4A, *TP53* and *DPC4/SMAD4* leads to a profound disturbance in cell cycle regulation, a hallmark of pancreatic cancer. Mutations in *K-RAS*, *INK4A*, *TP53* and *DPC4/SMAD4* are frequent, whereas mutations in the tumor suppressor *BRCA2*, mismatch repair genes and the serine-threonine kinases *AKT2* and *LKB1/STK11* are rare genetic events. Table 1 summarizes the reported frequencies of major genetic alterations in the pancreatic tumor progression model.

Genetic alterations with high frequency

Pancreatic cancer has the highest incidence of *RAS* mutations in human tumors identified to date [4]. The mutations of the *K-RAS* gene, *H-RAS* and *N-RAS* are not affected, are generally found in codon 12. Dependent on the used technique the frequencies of codon 12 mutations reported range from 20 to 100% and occur early in the tumor progression model [5,6]. The *RAS* family proteins encode small GTP-binding cytoplasmic proteins that mediate pleiotropic effects including cell proliferation, survival and migration [7]. Mutation of codon 12 in *K-*

Table 1: Frequency of major genetic alterations in pancreatic carcinoma.

Gene	Reference	Normal	PanIN1A	PanIN1B	PanIN2	PanIN3	Carcinoma
K-RAS	[6]	0%	38%	44%		87%	
	[36]	3%	30%	31%	73%		~90%
INK4a	[19]	0%	30%	27%	55%	71%	100%
	[14]		33%				40%
TP53	[37]	0%				12%	40%
	[35]	0%		35%		36%	40%
	[36]	0%	0%	0%	9,1%		87%
	[9]	0%	0%	0%	20%	57%	47%
DPC4/Smad4	[47]		0%	0%	0%	31%	55%
	[9]	0%	0%	0%	0%	33%	66%

RAS results in a gain of function, because the RAS protein remains trapped in the activated state. Considering RAS transforming potential, tissue and species differences come into question. In general epithelial human cells are not very sensitive to oncogene transformation. In contrast primary murine fibroblasts can be efficiently transformed by mutated RAS in concert with a second oncogene or loss of a tumor suppressor, like p53 or p16^{NK4a}. The sole expression of oncogenic RAS in primary rodent and human cells results in a permanent G1 arrest accompanied by accumulation of p53, p16^{INK4A} and p21^{CIP1} [8]. This senescence is thought to be a defense mechanism against oncogenic stress. Whether the observed overexpression of p21^{CIP1}, whose frequency parallels that of K-RAS Mutation in the pancreatic tumor progression model, is part of this defense mechanism or directly linked to the cell cycle by working as an assembly factor for the cyclin D1/CDK4 complex, is not known [9,10]. Placing p21^{CIP1} in a defence program is speculative but attractive, because it could explain in part the observation that oncogenic K-RAS mutations are not specific for malignancy, being present in benign diseases of the pancreas and in early clonal lesions. Moreover, the risk of progression to malignancy is low in the absence of co-operating genetic events [11–14]. Despite the high mutation frequency in human pancreatic carcinoma, mice which harbor a latent allele of K-Ras G12D capable of spontaneous activation in vivo, develop multiple early onset lung tumors but not pancreatic cancer, further demonstrating the species differences of RAS function [15]. The complexity of RAS function is amplified through recent data suggesting tumor suppressor properties of RAS. Transfection of wildtype Ras into rat fibroblasts inhibits anchorage-independent growth and colony formation, induced by the oncogenic Ras gene [16]. Furthermore – in vivo – Kras2 can inhibit lung carcinogenesis in mice [17]. A tumor suppressor function of K-RAS might also exist in the pancreas. Loss of the wildtype K-RAS allele was observed in some pancreatic

carcinoma cell lines with mutation in K-RAS (ASPC1, Capan1 and MiaPaca) and there was underexpression of the mutated allele in comparison to the wildtype allele in two other cell lines (Su8686 and Panc1) [5]. A further species difference affects the signaling pathway utilized by oncogenic RAS. Whereas in rodent cells the Raf/MAPK and the PI3K are thought to mediate many effects of oncogenic Ras, there are hints that in human cells the guanine nucleotide exchange factor Ral is sufficient for Ras transformation [18]. Therefore, the net outcome of RAS activation in a specific setting is not easy to predict and further studies, including primary cultures of epithelial pancreas cells, are needed.

Homozygous deletion of p16^{INK4A}/p14^{ARF} locus is a characteristic genetic alteration observed in 80% – 95% of human pancreatic cancer and usually occurs in later stages of the tumor progression model [19–21]. This locus on chromosome 9q21 encodes the two related tumor suppressor genes *INK4a* and *ARF*, whose coding sequence partially overlap. *INK4a* and *ARF* are generated by the use of a different first exon and an alternative reading frame in exon 2. Whereas *INK4a* regulates cell cycle progression as an inhibitor of the cyclin D/CDK 4/6 kinase complex, *ARF* directly interacts with Mdm 2/HDM 2 to block the interaction with p53 by localizing Mdm 2/HDM 2 to the nucleolus and by inhibiting directly Mdm 2/HDM 2's E3 ubiquitin ligase activity, contributing to p53 activation (figure 1) [22]. Gene deficient mice for p19^{Arf}, the mouse homologue of human p14^{ARF}, strongly suggest that *Arf* is the major tumor suppressor in mice [23,24]. The specific mutation of the *Ink4a* gene in mice also establishes p16^{Ink4a} as a tumor suppressor in mice [25,26]. Germline mutations in the exon 1α of *INK4a* are associated with the Familial Atypical Mole-Malignant Melanoma syndrome, implicating *INK4a* in human tumor susceptibility [27]. This mutation also predispose to pancreatic cancer, but in contrast to very high penetrance and early onset of

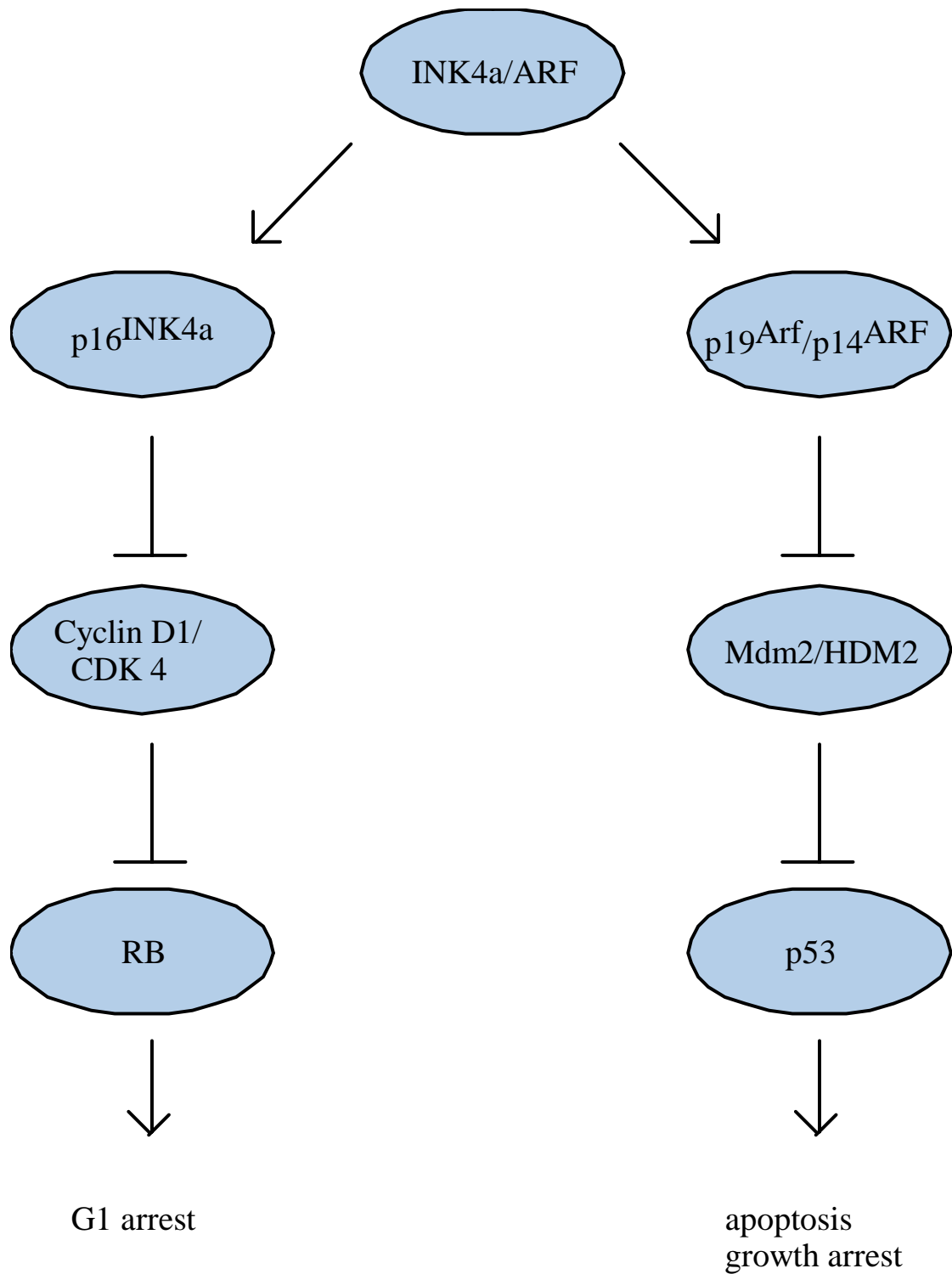


Figure 1

The *INK4a/ARF* locus. The two products of the *INK4a/ARF* locus encodes for p16^{INK4a} and p14^{ARF} (p19^{Arf} in mice). p16^{INK4a} indirectly regulates RB function and p14^{ARF} indirectly stabilizes p53.

melanoma, the penetrance of pancreatic cancer is very low and displays latency similar to the sporadic disease [28–30]. In humans *INK4a* seems to be the more important tumor suppressor for pancreatic cancer development, because germline and sporadic mutations have been identified that target *INK4a*, but omit *ARF* [21,31,32]. Mutations that selectively target *ARF* are rare findings [33]. In sporadic tumors *INK4a* is inactivated by homozygous deletions and intragenic mutation and in the remaining cases the *INK4a* gene is turned off through promoter methylation [20,34].

The *TP53* tumor suppressor gene is mutated, especially by missense mutations in sequences coding for the DNA binding domain, in greater than 50% of pancreatic adenocarcinomas. The mutations are often accompanied by loss of the wildtype allele and occur late in the progression model [21,35–39]. The transcription factor p53 regulates an essential growth checkpoint that both protects against genomic rearrangement or the accumulation of mutations, and suppresses cellular transformation caused by oncogene activation or the loss of tumor suppressor pathways. p53 is stabilized and activated by extracellular stress including γ irradiation and intracellular stress such as deregulation of cellular oncogenes. Once activated, p53 can induce cell cycle arrest or apoptosis [40]. Loss of p53 is associated with aneuploidy, an outstanding feature of pancreatic cancer, indicating that p53 function maintains genomic stability [41,42]. Germline mutations in *TP53* have been described as Li-Fraumeni syndrome, predisposing to several neoplasms, but pancreatic carcinomas are rare findings [43,44]. *SMAD4*, which was initially named *DPC4*, deleted in pancreatic carcinoma, was originally identified as a candidate tumor suppressor that is frequently inactivated in pancreatic tumors [45]. The transcription factor SMAD4 is an important regulator of the transforming growth factor β (TGF- β) signaling pathway [46]. Upon receptor activation SMAD proteins get phosphorylated and heterodimerize with Smad4 to transmit upstream signals to the nucleus and transactivate transcription of specific target genes. The *SMAD4* gene is deleted or mutated in over 50% of pancreatic carcinoma, an event occurring late in the tumor progression model [9,47]. The most prominent biological activity of TGF- β is its potent inhibition of cell growth, mediated by a cell cycle G1 arrest, in a wide variety of cells. It is assumed that the growth-inhibitory function of TGF- β is important for *SMAD4* tumor suppressor activity. But recent data also suggest TGF- β independent SMAD4 function. Restoration of *SMAD4* in human pancreatic carcinoma cells suppressed tumor formation in vivo and did not restore TGF- β sensitivity. Furthermore a decrease in pro-angiogenic VEGF expression and an increase in the angiogenesis inhibitor TSP-1 was observed, so that regulation of an angiogenic switch might contribute to the tumor suppressor

function of *SMAD4* [48]. Epigenetic inactivation of the TGF- β /SMAD4 pathway occurs in the presence of activated RAS, whereby explaining the reduced selection pressure for LOH at the *SMAD4* locus [49]. Recent studies suggest a more aggressive behavior of *SMAD4* negative pancreatic cancers [50,51].

Genetic alterations with low frequency

BRCA2 on chromosome 13q encodes for a protein that is needed for the maintenance of genomic stability by regulating DNA repair processes. Normal cells, deficient for *BRCA2*, accumulate lethal chromosomal aberrations [52]. Inherited *BRCA2* mutation predispose to early onset familiar breast and ovarian cancer [53]. Albeit with lower penetrance and equal age of onset, inherited *BRCA2* mutations also increase the risk for pancreatic cancer [54,55]. In sporadic pancreatic cancer *BRCA2* is inactivated in 7 to 10% and lately the biallelic inactivation in a high-grade duct lesion was demonstrated, so that it is assumed that *BRCA2* mutation occur late in the neoplastic progression in the pancreas [56].

The autosomal dominant inherited Peutz-Jeghers syndrome, caused by mutation of the serine-threonine kinase *LKB1/STK11* that maps to chromosome 19p13, is associated with an increased incidence of pancreatic carcinoma [57–60]. The signaling pathway of *LKB1/STK11* is so far unknown, but this gene was shown to be inactivated in 5% of sporadic pancreatic cancer, suggesting a possible role in tumor suppression [61,62].

The serine-threonine kinase *AKT2* is a candidate oncogene for human pancreatic cancer and was found to be amplified and overexpressed in pancreatic adenocarcinoma and cell lines in up to 20% [63–65]. *AKT2* is a downstream effector of the PI3 kinase and can be activated by epidermal growth factor, platelet-derived growth factor and basic fibroblast growth factor, all known to be overexpressed in pancreatic carcinoma [66,67]. Recently *AKT* signaling was linked to enforced insulin-like growth factor I receptor expression, promoting invasiveness of pancreatic cancer cells [68].

Autosomal dominant inherited Lynch syndrome is characterized by an increased risk of developing colorectal, endometrial, ovarian and breast cancers, transitional carcinoma of the ureter and renal pelvis [69]. Mutations in the DNA mismatch repair genes, including *hMLH1*, *hMSH2* and *hMSH6*, cause this syndrome. Pancreatic cancer is included in the tumor spectrum, however, it seems to be a rare finding [70,71]. Pancreatic cancers occurring in context of the Lynch syndrome are different compared to sporadic pancreatic carcinomas in terms of the superior clinical course, histopathology and distinct molecular genetic profiles, including retention of wildtype RAS [72–74].

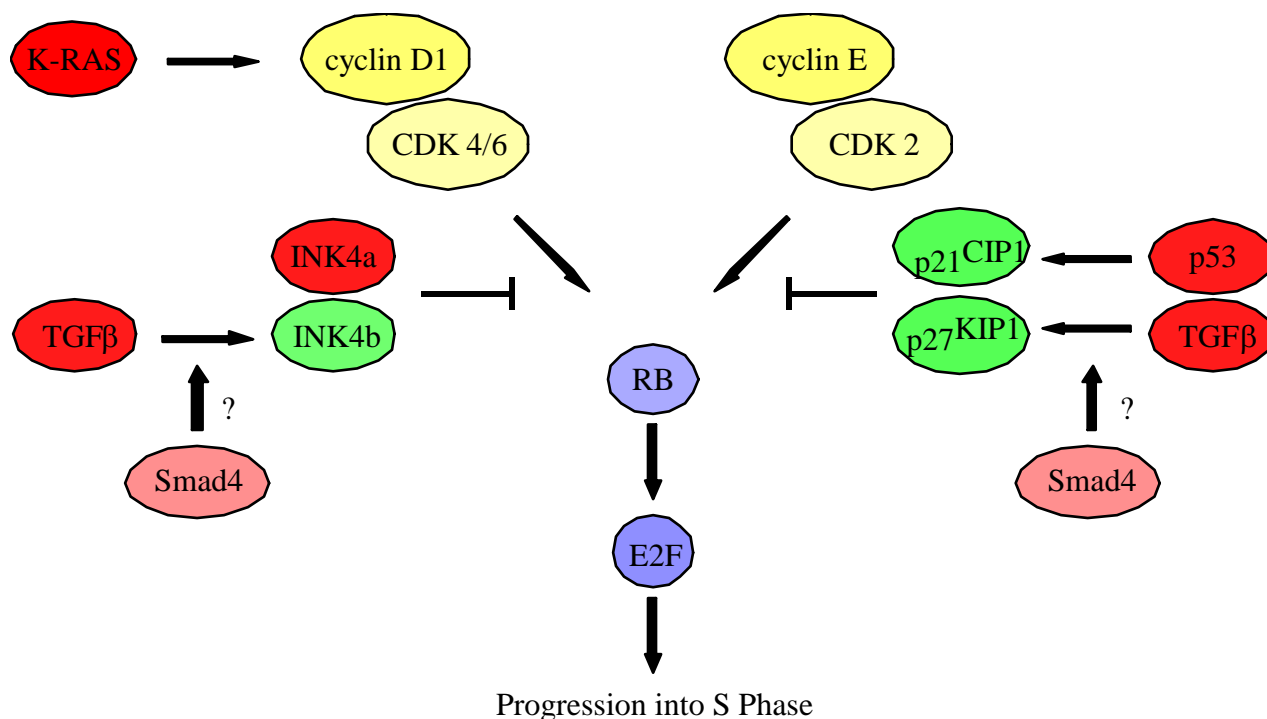


Figure 2

Interplay of the major genetic alteration in pancreatic carcinoma with the cell cycle. All four major genetic alteration, *K-RAS*, *INK4a*, *TP53* and the *TGF-β/SMAD4* tumor suppressor pathway, observed in pancreatic carcinoma, regulate directly or indirectly G1 progression, leading to E2F dependent S phase entry.

Furthermore, microsatellite instability is unlikely to participate in the oncogenesis of spontaneous pancreatic cancer [75–77].

Conclusion

Molecular investigations of pancreatic cancer are complicated by the restricted accessibility of the organ for biopsies. The findings in molecular research on pancreatic carcinoma of the last years is now integrated in a pancreatic tumor progression model, with genetically, epigenetically and morphological defined precursor lesions. Pancreatic cancer is a genetic disease, but the transition between cancer and non-cancer is not regulated by a simple switch activated by a single gene. In fact, multiple mutations must accumulate in a single cell, including overexpression of receptor-ligand systems, oncogene activation and loss of tumor suppressor genes, to develop pancreatic carcinoma. But where are the diverse genetic alterations, whose number is increasing, integrated? Deregulated cell cycle is the hallmark of many human tumors, including pancreatic carcinoma, and therefore the cell cycle could be placed into the center of pancreatic oncogen-

esis. As figure 2 illustrates, each of the major genetic alteration mentioned above is involved in cell cycle regulation and leads together to the acceleration of the cell cycle progression and continuous growth.

Further analysis of the underlying molecular mechanism will offer new diagnostic and therapeutic options and, hopefully improve the outcome of this dismal disease in the future.

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