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ORIGINAL ARTICLE

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Genetic profile of 22 pancreatic carcinoma cell lines

Analysis of K-*ras*, *p53*, *p16* and *DPC4/Smad4*

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Abstract The K-*ras*, *p53*, *p16* and *DPC4* genes are among those most frequently altered in pancreatic ductal carcinoma. We analyzed 22 cell lines for alterations in these genes by direct sequence analysis and methylation-specific polymerase chain reaction. These cell lines showed mutations in K-*ras* and *p53* at frequencies of 91% and 95%, respectively. Alterations in *p16*^{INK4a} were found in all cases and included nine homozygous deletions, seven mutations and promoter methylation in six cases. Eight cell lines (36%) had an alteration of *DPC4*, including one mutation and seven homozygous deletions. The most typical mutational profile involved K-*ras*, *p53*, and *p16*^{INK4a}, concurrently aberrated in 20 cases (91%). Eight cell lines had alterations in all four genes. Inactivation of *DPC4* was always accompanied by alteration of all of the other three genes. This comprehensive data regarding the cumulative genetic alterations in pancreatic carcinoma cell lines will be of great value for studies involving drug sensitivity or resistance that may be associated with inactivation of a particular gene or molecular pathway.

Keywords Pancreas · Carcinoma cell lines · K-*ras* · *P16* · *P53* · *DPC4/Smad4*

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Introduction

Information regarding the mutational status of multiple genes in cancer will aid not only in a greater understanding of the molecular processes involved in tumorigenesis but may also be valuable in the design of molecular diagnosis strategies, in studies in which certain genetic alterations may be related to drug resistance and in the molecular epidemiology of pancreatic cancer. This would therefore have dramatic implications for therapeutic intervention. One such example is the recent demonstration that the low efficacy of 5-fluorouracil is causally related to the inactivation of the *p53* gene by mutation [4, 22].

Pancreatic ductal adenocarcinoma represents a unique opportunity to study multigenic mutational status, as it is characterized by a relatively unique molecular fingerprint comprising activating point mutations at codon 12 of the K-*ras* oncogene in 80% of cases and inactivation of the tumor suppressor genes *p16*$^{INK4a/(CDKN2/MTS1)}$ and *p53* in 90% and 60% of cases, respectively [1, 5, 19, 25, 32, 35, 36, 37, 41]. More recently, the *DPC4/Smad4* gene has been reported to be altered in about 50% of xenografted cancers [11].

Molecular studies in primary ductal carcinoma of the pancreas are problematic because of the tumor's conspicuous desmoplastic stroma, which makes the isolation of cells difficult. Such studies are therefore much easier to perform in tumor cell lines and xenografts [42]. While the former are easily handled, the latter are not readily

available to all researchers, as they require special and costly facilities. The use of both cell lines and xenografts is limited by the potential acquisition of additional mutations by tumor cells during their manipulation [33]. Cell lines nonetheless represent a commonly used source of material, and some of these have been characterized for a number of different chromosomal and gene anomalies. While data regarding the cumulative genetic alterations in pancreatic carcinoma xenografts have been reported [34], a comprehensive analysis of commonly used pancreatic ductal carcinoma cell lines has not been performed. In this report, we present the results of the analysis of 22 pancreatic cancer cell lines for alterations in the K-*ras*, *p53*, *p16* and *DPC4* genes.

Materials and methods

Cell lines

A total of 24 human pancreatic ductal carcinoma cell lines were originally analyzed in this study (Table 1). Two cell lines, SW850 and SW979, were excluded, because a recent analysis revealed that these cell lines most likely are derived from cervix carcinomas. In particular, testing of the original cell lines received from Memorial Sloan-Kettering Cancer Center in the mid 1980s revealed that SW850 and SW979 contain the same HPV16 sequences as the cervix carcinoma cell lines C4–1 and C4–2 (H. Kalthoff, personal communication). Because of these findings – the fact that they lack any K-*ras*, *p53*, *p16* and *DPC4* mutations and the uncertain documentation of their origin – these cell lines should not be regarded as pancreatic carcinoma cell lines. All cell lines were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (Gibco BRL, Life Technologies) and were *Mycoplasma* free.

Analysis of K-*ras*, *p16*, *p53* and *DPC4*

All samples were analyzed for mutations in exon 1 of the K-*ras* gene, exons 1 and 2 of *p16*, exons 5–9 of *p53* and exons 8–11 of *DPC4* by direct sequencing of polymerase chain reaction (PCR)-amplified DNA fragments. Primers for amplification of the *p53* [36], *p16* [49], K-*ras* [37] and *DPC4* genes [12] were as described. Methylation-specific PCR for the 5′ CpG island of the *p16* gene was carried out as described [13].

H. Kalthoff, personal communication

Testing of the original cell lines 1983, received from Dr. Jørgen Fogh, Sloan-Kettering Institute for Cancer Research, revealed that SW850 and SW979 contain the same HPV18 sequences as the cervix carcinoma cell line C4-I (E. Schwarz, DKFZ, Heidelberg; J. Schwarte-Waldhoff, IML, Bochum). Because of these findings, the fact that they lack any K-*ras*, *p53*, *p16* and *DPC4* mutations and the uncertain documentation of their origin, these cell lines should not be regarded as pancreatic carcinoma cell lines.

Results and discussion

The 22 cell lines were analyzed for mutations in exon 1 of K-*ras*, exons 5–9 of *p53* and in highly conserved exons 8–11 of *DPC4*. The *p16* gene was examined for alterations in exons 1 and 2 as well as for methylation of its 5′ CpG island by methylation-specific PCR. The results are summarized in Table 2. Activating mutations in K-*ras* at codon 12 were found in 20 samples (91%); twelve of these were G to A transitions. Inactivating mutations of *p53* were found in 21 of 22 (95%) cases, with G to A transitions being the most common type of nucleotide change (6 of 21, 28.5%). The *p16* gene was altered

Table 1 Origin of pancreatic ductal carcinoma cell lines

Cell line	Synonym (misnomer)	Established by	Source of tumor cells	Provided by
A818–4		H. Kalthoff (Germany)	Ascites	H. Kalthoff
ASPC-1		Chen and M.H Tan (USA) [6]	Ascites	American Type Culture Collection
BI		A. Andren-Sandberg (Sweden)[a]		A. Andren-Sandberg
BJ		A. Andren-Sandberg (Sweden)[a]		A. Andren-Sandberg
CFPAC1		R.A. Schoumacher (USA) [38]	Liver metastasis	N. Lemoine
FA6		N. Nagata (Japan) [29]		N. Lemoine
Ger		A.G. Grant (Great Britain) [9]	Primary tumor	N. Lemoine
HPAF-II		R.S. Metzgar (Germany) [28]	Ascites	N. Lemoine
IMIM-PC2		M. R. Vila (Spain) [44]	Primary tumor	F.X. Real
MDAPanc3		M. Frazier (USA) [8]	Liver metastasis	N. Lemoine
MiaPaCa-2		A. Yunis (USA) [48]	Primary tumor	American Type Culture Collection
Paca3	Pc3	M. v Bülow (Germany)[b]	Primary tumor	M. v Bülow
Paca44	Patu8902	M. v Bülow (Germany)[b]		M. v Bülow
Panc1		M. Lieber (USA) [26]	Primary tumor	American Type Culture Collection
PancTuI	PaCa2 (originally by M. Bülow) PancTuII PaTu-I Panc2, Pc2	M. v Bülow (Germany)[b]	Primary tumor	M. v Bülow
PC		A. Andren-Sandberg (Sweden)[a]		A. Andren-Sandberg
PSN1		H. Yamada (Japan) [47]	Primary tumor	N. Lemoine
PT45P1		H. Kalthoff (Germany)	Primary tumor	H. Kalthoff
RWP1		D.L. Dexter, P. Calabresi (USA) [7]	Liver metastasis	N. Lemoine
SK-PC1		M. R. Vila (Spain) [44]	Primary tumor	F.X. Real
SUIT-2		T. Iwamura (Japan) [17]	Liver metastasis	T. Iwamura
T3M4	Panc89	T. Okabe (Japan) [31]	Lymph node metastasis	N. Lemoine

[a] A. Andren-Sandberg, personal communication
[b] G. Klöppel, personal communication

Table 2 Molecular alterations of K-*ras*, *p53*, *p16* and *DPC4* in pancreatic ductal carcinoma cell lines. *HD* homozygous deletion, *Wt* wild type

	K-*ras*		*p53*		*p16*		*DPC4*		No. of genes mutated
	Alteration	Predicted product	Alteration	Predicted product	Alteration	Predicted product	Alteration	Predicted product	
A818.4	12 GGT-CGT	Gly to arg	Mutated in tetramerization domain *	Shorter protein	HD	Absent	None	Wt	3
AsPc1**	12 GGT-GAT	Gly to asp	135 TGC-GC	Frameshift	77 ACT-A	Frameshift	None	Wt	3
BI	12 GGT-GAT	Gly to asp	197 GTG-TTG	Val to leu	Methylated	Absent	HD	Absent	4
BJ	12 GGT-GAT	Gly to asp	275 TGT-TAT	Cys to tyr	44 TAC-TAAC	Tyr to stop	HD	Absent	4
CFPAC1	12 GGT-GTT	Gly to val	242 TGC-CGC	Cys to arg	Methylated	Absent	HD	Absent	4
FA6***	12 GGT-GAT	Gly to asp	149, 840bp del	Truncated	58 CGA-TGA	Arg to stop	HD	Absent	4
Ger	12 GGT-GAT	Gly to asp	272 GTG-TTG	Val to leu	HD	Absent	HD	Absent	4
HPAF II	12 GGT-GAT	Gly to asp	151 CCC-TCC	Pro to ser	29–34 del	In-frame deletion	None	Wt	3
IMIM-PC2	12 GGT-GAT	Gly to asp	306 CGA-TGA	Arg to stop	HD	Absent	None	Wt	3
MDAPanc3	12 GGT-GCT	Gly to ala	273 CGT-TGT	Arg to cys	–36 to (+5)-C	Absent	None	Wt	3
MiaPaCa2	12 GGT-TGT	Gly to cys	248 CGG-TGG	Arg to trp	HD	Absent	None	Wt	3
PaCa3	None	Wt	None	Wt	Methylated	Absent	None	Wt	1
PaCa44	12 GGT-GTT	Gly to val	176 TGC-AGC	Cys to ser	Methylated	Absent	None	Wt	3
PANC1	12 GGT-GAT	Gly to asp	273 CGT-CAT	Arg to his	HD	Absent	None	Wt	3
PancTu-I	12 GGT-GTT	Gly to val	176 TGC-AGC	Cys to ser	Methylated	Absent	None	Wt	3
PC	12 GGT-GTT	Gly to val	175 CGC-CAC	Arg to his	HD	Absent	355 GAC-GGC	Asp to gly	4
PSN1	12 GGT-CGT	Gly to arg	132 AAG-CAG	Lys to gln	HD	Absent	HD	Absent	4
PT45P1	13 GGC-GAC	Gly to asp	280 AGA-AAA	Arg to lys	HD	Absent	None	Wt	3
RWP1	12 GGT-GAT	Gly to asp	175 CGC-CAC	Arg to his	58 CGA-TGA	Arg to stop	None	Wt	3
SK-PC 1	12 GGT-GAT	Gly to asp	282 CGG-CTG	Arg to leu	HD	Absent	HD	Absent	4
Suit-2	12 GGT-GAT	Gly to asp	273 CGT-CAT	Arg to his	69 GAG-TAG	Glu to stop	None	Wt	3
T3M4	None	Wt	220 TAT-TGT	Tyr to cys	methylated	Absent	None	Wt	2

* Personal communication from H. Kalthoff
** AsPc1 is reported to have a mutation in exon 2 of DPC4 (24)
*** FA6 also has the A148T polymorphism in p16

in all of the cell lines; homozygous deletions were seen in nine (41%) tumors, seven (32%) contained mutations or small deletions, while six other cell lines (27%) had methylation of the 5′ CpG island. For the cases that showed methylation, transcriptional inactivation of the *p16* gene was verified by reverse-transcription (RT)-PCR (data not shown). Homozygous deletion of the *DPC4* gene was observed in seven cell lines (32%), while only one mutation was found (4.5%). Thus, 36% of cases had alterations of *DPC4*. All eight cell lines showing abnormalities in *DPC4* had concurrent alteration of all the other three genes analyzed.

Pancreatic ductal adenocarcinomas share the high rate of K-*ras*, *p53* and *p16* alterations with a number of cancers from other organs [2, 3, 11, 12, 16, 20, 21, 23, 24, 27, 30, 34, 39]. *DPC4* changes, however, are rarely seen in extrapancreatic cancers (except for colorectal carcinomas) at the same high frequency as in pancreatic ductal carcinoma [34, 43]. The reported frequency of homozygous deletion of *DPC4* in cell lines and xenografts from ductal adenocarcinoma ranges from 20% to 53% [11, 45]. *DPC4* mutations, in contrast, were either not found [3] or were detected at a much lower frequency (16%) [34]. These data compare well with our results. Since only one study found that *DPC4* mutations (4 of 12 short-term cultured pancreatic carcinoma cell lines) were more frequent than homozygous deletions (1 of 12) [18], it seems that homozygous deletion is the preferred inactivation mechanism for *DPC4* in pancreatic carcinoma. Compared with other genetic changes in pancreatic ductal carcinoma, loss of heterozygosity on chromosome 18q is one of the most frequent genetic events [40]. Given the fact that these deletions are usually very large and may involve the entire chromosome [10], there may be additional deletional targets in addition to *DPC4*. This possibility has been explored in more detail in recent reports [14, 15].

The frequencies of genetic alteration for each individual gene are those largely expected from existing data. Multigenic analysis showed that 20 of the 22 cell lines (91%) had concurrent alterations in K-*ras*, *p53* and *p16*. These patterns of accumulated gene inactivation are detailed in Table 3. A previous multigenic analysis of 41 xenografts observed mutation of K-*ras* in all samples [34]. A trend was also seen between mutation of *DPC4* and *p16* in that alteration of the former was always accompanied by alteration of the latter [34]. In this panel of cell lines, alteration of *DPC4* was only seen in those cases having alterations in all three of the other genes. This might indicate that alteration of *DPC4* is a late pathogenetic event, a possibility further suggested by a recent study [46].

In summary, the molecular alterations present in this series of cell lines represent the variety of alterations

Table 3 Accumulation of genetic alterations in pancreatic ductal carcinoma cell lines

Altered genes	Number of affected cell lines
K-*ras*, *p53*, *p16*, *DPC4*	8
K-*ras*, *p53*, *p16*	12
p*53*, *p16*	1
p16	1

present in primary pancreatic carcinomas. The comprehensive data regarding the multigenic alterations in this large series of cell lines should prove valuable for studies involving drug sensitivity or resistance that may be associated with inactivation of a particular gene or molecular pathway.

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