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Advances in Brief

DPC4 Gene in Various Tumor Types[1]

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Abstract

We recently identified a novel tumor-suppressor gene, *DPC4*, at chromosome 18q21.1 and found that both alleles of *DPC4* were inactivated in nearly one-half of the pancreatic carcinomas. Here, we analyzed 338 tumors, originating from 12 distinct anatomic sites, for alterations in the *DPC4* gene. Sixty-four specimens were selected for the presence of the allelic loss of 18q and were further analyzed for *DPC4* sequence alterations. An alteration of the *DPC4* gene sequence was identified in one of eight breast carcinomas and one of eight ovarian carcinomas. These results indicate that whereas *DPC4* inactivation is prevalent in pancreatic carcinoma (48%), it is distinctly uncommon (<10%) in the other tumor types examined. The tissue restriction of alterations in *DPC4*, as in many other tumor-suppressor genes, emphasizes the complexity of rate-limiting checkpoints in human tumorigenesis.

Introduction

Allelotype analysis of pancreatic carcinoma has indicated that about 90% of these tumors show allelic loss of chromosome 18q (1). We recently identified the *DPC4* gene (for deleted in pancreatic carcinoma, locus *4*) as a genetic target of these losses (2). *DPC4* was homozygously deleted in about 30% of pancreatic carcinomas and inactivated by intragenic mutation in another 20% of the tumors.

A variety of tumor types exhibit allelic loss of 18q. To survey the involvement of *DPC4* in different tumor types, we analyzed 338 tumors from outside of the gastrointestinal tract for *DPC4* gene alterations. Sixty-four specimens were selected for 18q loss and high neoplastic cellularity and were further analyzed for alterations in the *DPC4* gene sequence.

Materials and Methods

Tumor Samples. Seventy-three of 347 tumor samples were selected for allelic loss of chromosome 18q21 and high neoplastic cellularity. All selected tumor samples are listed in Table 1. The tumor set included bladder, breast, head and neck, hepatocellular, lung, ovarian, prostatic, and renal cell carcinomas, glioblastomas and medulloblastomas, melanomas and osteosarcomas, and nine additional pancreatic carcinomas. The six lung carcinomas included one carcinoid, three small cell lung carcinomas, and two non-small cell lung carcinomas; all three primary ovarian carcinomas were serous carcinomas. Fourty-one of the specimens were primary tumors; 24 were tumor cell lines; and 8 were xenografts.

PCR and Sequencing. Microsatellite analysis and PCR were performed in microtiter plates as described (1, 3). PCR reactions were incubated with 10 units of Exonuclease I and 2 units of shrimp alkaline phosphatase (United States Biochemical Corp., Cleveland, OH) in a final volume of 50 μl PCR buffer for 15 min at 37°C and 15 min at 80°C. Sequencing of 5 μl enzyme-treated PCR product was performed in microtiter plates by Sequitherm cycle sequencing, according the recommendations of the manufacturer (Epicentre Technologies, Madison, WI). PCR and sequencing primers are available on the Internet (http://www.med.jhu.edu/pancreas/index.htm).

Results and Discussion

Sixty-four cancers from outside of the gastrointestinal tract and nine pancreatic carcinomas were analyzed for *DPC4* gene alterations. The tumors were selected from a series of 347 neoplasms for the presence of allelic loss of 18q, as determined by microsatellite analysis using the markers *D18S46, D18S363,* and *D18S474* (Table 2; Ref. 4). True LOH[3] had been determined for the bladder, head and neck, and prostatic carcinomas as part of previous studies by comparison of tumor DNA with constitutional normal DNA. The other specimens were selected on the basis of statistical evidence for LOH, as determined by the presence of a single allele size at each of the three loci in the tumor DNA. With a heterozygosity value of >0.7 for each marker, this selection reflects presumptive LOH, with an estimated $P < 0.03$. Finally, only the tumor samples that had high neoplastic cellularity, as judged by a decrease in allele intensity of at least 50% in the microsatellite analysis, were selected for *DPC4* sequence analysis.

The 11 exons of *DPC4* were amplified by PCR and sequenced directly by cycle sequencing. The breast carcinoma cell line MDA-MB468 was found to have a homozygous deletion of the complete coding sequence of *DPC4,* whereas the flanking microsatellite markers *D18S46, D18S363,* and *D18S474* were retained. The pancreatic carcinoma cell line Colo357 had a homozygous deletion involving exons 1–4 of *DPC4*, whereas the remaining exons were retained. Duplex PCRs for exons 1 and 10 of *DPC4* and the *DPC1* locus at 13q (3) confirmed both homozygous deletions and ensured DNA quality (Fig. 1*A*). Sequence analysis of *DPC4* revealed alterations in the ovarian carcinoma cell line SW626, the pancreatic carcinoma cell lines AsPc1 and Capan1, and the pancreatic carcinoma xenograft MX36 (Fig. 1*B* and Table 3). The alterations in SW626 and AsPc1 predicted nonconservative amino acid replacements (Asp → His and Arg → Thr, respectively), whereas the alterations in Capan1 and MX36 predicted truncations of the protein (a nonsense codon and a 2-bp frameshift, respectively). The mutations were confirmed by sequencing of a second independently amplified PCR product. The constitutional normal DNAs for the tumors with mutations were not available to determine whether the alterations were somatically acquired or present in the germline. Analysis of more than 100 chromosomes, however, had not identified these sequence alterations, rendering them unlikely to be common sequence polymorphisms.

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[3] The abbreviation used is: LOH, loss of heterozygosity.

Table 1 *Tumor samples analyzed for DPC4 gene alterations*

The tumor set selected for *DPC4* sequencing was based on the presence of allelic loss at 18q21 and high neoplastic cellularity.

Tumor type	Sample	Source[a]	Origin[b]
Bladder carcinoma	BT3	P	A
Bladder carcinoma	BT4	P	A
Bladder carcinoma	BT6	P	A
Bladder carcinoma	BT7	P	A
Bladder carcinoma	BT8	P	A
Bladder carcinoma	BT10	P	A
Bladder carcinoma	MX19	X	A
Breast carcinoma	BT483	L	ATCC
Breast carcinoma	BT549	L	ATCC
Breast carcinoma	MCF7	L	ATCC
Breast carcinoma	MDA-MB415	L	ATCC
Breast carcinoma	MDA-MB436	L	ATCC
Breast carcinoma	MDA-MB468	L	ATCC
Breast carcinoma	T47D	L	ATCC
Breast carcinoma	ZR75–30	L	ATCC
Glioblastoma	BX271	X	B
Glioblastoma	BX368	X	B
H&N carcinoma[c]	38T	P	A
H&N carcinoma	225T	P	A
H&N carcinoma	243T	P	A
H&N carcinoma	MX32	X	C
H&N carcinoma	MX47	X	C
Hepatocellular carcinoma	L3	P	D
Hepatocellular carcinoma	L9	P	D
Hepatocellular carcinoma	L10	P	D
Hepatocellular carcinoma	L14	P	D
Hepatocellular carcinoma	L16	P	D
Hepatocellular carcinoma	L18	P	D
Lung carcinoma	H157	P	E
Lung carcinoma	H249	P	E
Lung carcinoma	H727	P	E
Lung carcinoma	N417	P	E
Lung carcinoma	OH1	P	E
Lung carcinoma	MX44	X	C
Medulloblastoma	BX341	X	B
Melanoma	HM + 86	P	F
Melanoma	M91–054	P	F
Melanoma	UACC827	P	F
Melanoma	UACC1022	P	F
Osteosarcoma	Os1	P	D
Osteosarcoma	Os6	P	D
Osteosarcoma	Os7	P	D
Ovarian carcinoma	SO3T	P	G
Ovarian carcinoma	SO6T	P	G
Ovarian carcinoma	SO9T	P	G
Ovarian carcinoma	CaOv3	L	ATCC
Ovarian carcinoma	CaOv4	L	ATCC
Ovarian carcinoma	NIH-OvCar3	L	ATCC
Ovarian carcinoma	SKOV3	L	ATCC
Ovarian carcinoma	SW626	L	ATCC
Pancreatic carcinoma	AsPc1	L	ATCC
Pancreatic carcinoma	Capan1	L	ATCC
Pancreatic carcinoma	Capan2	L	ATCC
Pancreatic carcinoma	Colo357	L	ECACC
Pancreatic carcinoma	Miapaca2	L	ATCC
Pancreatic carcinoma	Panc1	L	ATCC
Pancreatic carcinoma	PL45	L	C
Pancreatic carcinoma	Su8686	L	ATCC
Pancreatic carcinoma	MX36	X	C
Prostatic carcinoma	13T	P	H
Prostatic carcinoma	25T	P	H
Prostatic carcinoma	47T	P	H
Prostatic carcinoma	51T	P	H
Prostatic carcinoma	128T	P	H
Prostatic carcinoma	142T	P	H
Prostatic carcinoma	402T	P	H
Prostatic carcinoma	412T	P	H
Prostatic carcinoma	DU145	L	ATCC
Prostatic carcinoma	LNCaP	L	ATCC
Prostatic carcinoma	PC3	L	ATCC
Renal cell carcinoma	K2	P	D
Renal cell carcinoma	K3	P	D
Renal cell carcinoma	K5	P	D

[a] P, primary tumor; L, cell line; X, xenograft.

[b] Tumor samples were derived from ATCC, American Type Culture Collection; or ECACC, European Collection of Animal Cell Cultures; or obtained from sample banks of: A, David Sidransky; B, Bert Vogelstein; C, Scott E. Kern; D, Ralph H. Hruban; E, Robert A. Cassero, Jr.; F, Paul S. Meltzer; G, Lora Hedrick and Kathleen R. Cho; and H, G. Steven Bova and William B. Isaacs.

[c] H&N, head and neck.

Table 2 *Allelic loss of 18q in various tumor types*

Tumor type	18q loss[a] (this study) *n* %	18q loss[b] (literature) % (Ref.)	No. selected for sequencing[c]
Bladder transitional cell carcinoma	10/83 12	12 (5), 35 (6)	7
Breast carcinoma	8/22 36	8 (7), 24 (8), 31 (9), 35 (10)	8
Glioblastoma	2/20 10		2
Head/neck squamous cell carcinoma	14/50 28	23 (11), <5 (12), 8 (13), 25 (14),31 (15)	5
Hepatocellular carcinoma	6/25 24	9 (16)	6
Lung carcinoma	6/17 35	24 (17), 65 (18), 14 (19)	6
Medulloblastoma	1/10 10		1
Melanoma	4/18 22	22 (20)	4
Osteosarcoma	3/13 23	64 (21), 18 (22)	3
Ovarian carcinoma	8/12 67	47 (23), 29 (24), 27 (25)	8
Pancreatic carcinoma	9/9 100	89 (1)	9
Prostatic carcinoma	14/46 30	45 (26), 26 (27), 19 (28)	11
Renal cell carcinoma	3/22 14	<5 (29), <5 (30)	3
Total			73

[a] Percentages reflect true LOH or presumptive LOH (see text).

[b] Data were derived from the indicated references.

[c] Number of tumor samples after selection for 18q21 allelic loss and high neoplastic cellularity. Some primary tumors that scored as having LOH did not meet the requirements for sequencing, for technical reasons. The tumors are listed individually in Table 1.

Three of the sequence alterations identified in this series were in exon 8, within 25 bp of each other, and one was in exon 2 (Table 3). Although data are limited, the locations of the *DPC4* sequence changes suggest mutational hotspots in exons 8 and 11; 4 of the 11 currently known sequence alterations are in exon 8, and another 4 are in exon 11 (2; this study).[4] Of note, the regions of strongest homology between *DPC4* and the *D. melanogaster Mad* and *C. elegans Sma2* genes include these putative mutational hotspots (2).

We previously reported that *DPC4* was inactivated in 20 of 41 pancreatic carcinoma xenografts (2). These inactivations included 14 homozygous deletions and six intragenic alterations. The identification here of one homozygous deletion and three intragenic alterations in nine pancreatic carcinoma cell lines further substantiates the mutational involvement of *DPC4* in pancreatic carcinoma. Together, 24 (48%) of 50 pancreatic carcinomas examined have been found to have mutational inactivations of *DPC4*.

We previously reported a homozygous deletion in one of two bladder carcinoma xenografts (2). Here, we sequenced the second xenograft and six primary bladder carcinomas but did not identify additional alterations in *DPC4*. It should be noted that the detection of homozygous deletions in primary tumors by standard PCR is generally hampered by the presence of nonneoplastic cells (31). Forty-one of the 73 tumors analyzed here were primary tumors (Table 1), potentially impairing the detection of homozygous deletions in these specimens.

Our data indicated that *DPC4* gene alterations are restricted to tumors arising in specific types of tissue. Many of the tumor types examined exhibit rather low frequencies of 18q LOH, and the two *DPC4* alterations identified in nonpancreatic tumors were in cancers that exhibit moderate or high LOH of 18q (Table 2). However, all tumors tested were selected for 18q LOH; yet, only two alterations were identified in 64 tumors arising outside the gastrointestinal tract. This suggests that other tumor-suppressor gene(s) might be targets of the 18q losses. Analysis of the candidate tumor-suppressor gene *DCC* at 18q has been difficult, due to its size and complexity (32).

Allelotype analyses have suggested that frequent alterations of a rather restricted set of tumor-suppressor genes are likely to be of

[4] A. T. M. S. Hoque and S. E. Kern. Mutational involvement of DPC4 in colitis-associated neoplasia, submitted for publication.

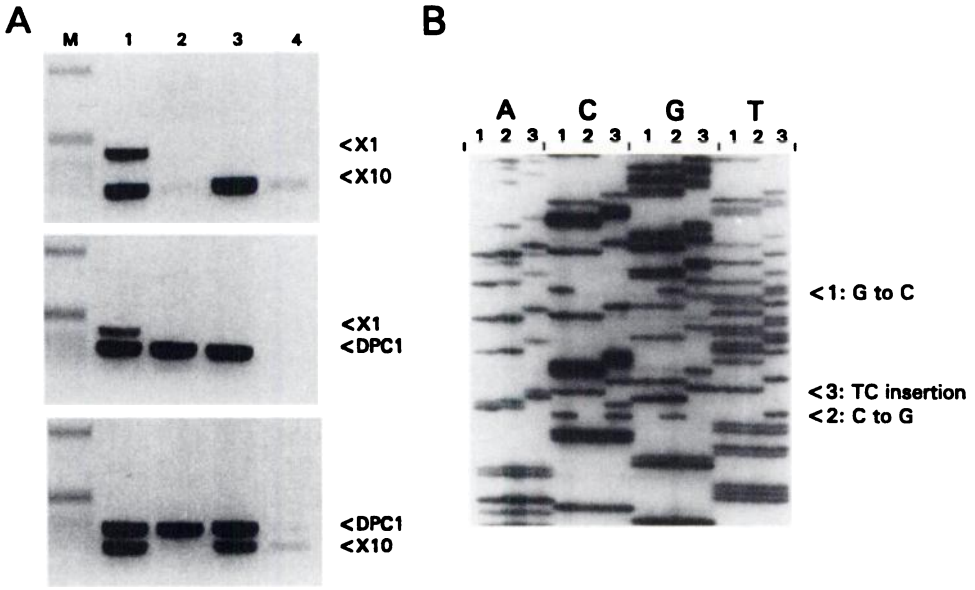

Fig. 1. *A*, Duplex PCR analysis of homozygous deletions involving the *DPC4* gene. *Top panel*, duplex PCR for exons 1 and 10 of *DPC4*; *middle panel*, PCR for exon 1 of *DPC4* and the *DPC1* locus at 13q; *bottom panel*, PCR for exon 10 of *DPC4* and *DPC1*. *M*, 1-kb ladder (Life Technologies, Inc.); *Lanes: 1*, normal DNA serving as a positive control; *2*, breast carcinoma cell line MDA-MB468, which had a homozygous deletion involving the complete coding sequence of *DPC4*; *3*, pancreatic carcinoma cell line Colo357, which had a homozygous deletion involving exons 1–4 of *DPC4*; *4*, template-negative control. *B*, sequence analysis of mutations in exon 8 of the *DPC4* gene. *Lanes: 1*, ovarian carcinoma cell line SW626, a GAT → CAT missense mutation; *2*, pancreatic carcinoma cell line Capan1, a TCA → TGA nonsense mutation; *3*, pancreatic carcinoma xenograft MX36, a TC insertion.

Table 3 *DPC4 alterations*

Tumor	Tissue	Alteration	Codon	Exon	Predicted effect
MDA-MB468	Breast	homozygous deletion		1–11	No protein
SW626	Ovarian	GAT → CAT	351	8	Asp → His
Colo357	Pancreas	homozygous deletion		1–4	No protein
AsPc1	Pancreas	AGG → ACG	100	2	Arg → Thr
Capan1	Pancreas	TCA → TGA	343	8	Ser → Stop
MX36	Pancreas	TCA → TCTCA	343	8	Frameshift

DPC4 alterations identified in this study in a set of 73 tumor samples. Early studies had identified genetic inactivation of *DPC4* in nearly one-half of pancreatic carcinoma xenografts, three pancreatic carcinoma cell lines (BxPc3, CFPAC1, and HS766T), two colorectal, one biliary, and one bladder carcinoma, and an ulcerative colitis-associated dysplasia (2).[4]

major importance for most tumor types (1, 5–30). A set of inactivated tumor-suppressor genes appears to be characteristic for a particular tumor type and can be distinctive even for tumors that arise in related anatomical sites. Frequent inactivation of the *APC* gene, for example, is characteristic of colorectal carcinomas (33) but not for pancreatic carcinomas (34–36). Vice-versa, the *p16* gene is frequently inactivated in pancreatic carcinomas (37) but not in colorectal carcinomas (38). Indeed, the broad spectrum of tumors that harbor *p53* alterations might be the exception among tumor-suppressor genes (39). The importance of genes that sustain low-prevalence alterations, however, may as yet be underestimated. Such events may contribute significantly to the genetic variety within a tumor type and, thus, to the complexity of human tumorigenesis. Low-prevalence alterations would become increasingly important if multiple alterations of this type accumulated in individual tumors. Allelotype analyses have indeed suggested that this is likely to be the case (1, 5–30).

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