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Advances in Brief

Frequent Loss of Expression and Loss of Heterozygosity of the Putative Tumor Suppressor Gene *DCC* in Prostatic Carcinomas[1]

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Abstract

The putative tumor suppressor gene *DCC* has been shown to be frequently lost or expressed at low levels in colorectal, gastric, pancreatic, and esophageal carcinomas. In the present study, the *DCC* gene and its mRNA expression in human and rat prostatic carcinoma cells as well as in prostatic carcinoma tissues were examined by reverse transcriptase-polymerase chain reaction and polymerase chain reaction-loss of heterozygosity. The *DCC* gene was present and expressed in normal prostatic cells. However, its expression was decreased or undetectable in all prostatic carcinoma cells from either humans (4 cell lines) or rats (5 cell lines). In patients, 12 of 14 cases (86%) showed reduced *DCC* expression and 5 of 11 informative cases (45%) showed loss of heterozygosity at the *DCC* locus. These results demonstrate that loss of *DCC* expression and loss of heterozygosity at the *DCC* locus are a frequent feature of prostatic carcinoma cells.

Introduction

Inactivation of tumor suppressor genes has been shown to play an important role in the development of a variety of human cancers (1, 2). The mechanisms of inactivation include allelic deletion, chromosomal rearrangement, point mutation, and binding of suppressor gene products with viral or cellular inactivating proteins (1–3). To date, several tumor suppressor genes have been discovered, which include but are not limited to the retinoblastoma susceptibility (*RB*), *p53*, Wilm's tumor (*WT-1*), neurofibromatosis type 1 (*NF-1*), mutated in colorectal cancer (*MCC*), adenomatous polyposis coli (*APC*), and *DCC*[3] genes (1, 2, 4–6). The *DCC* gene has been partially cloned and deduced amino acid sequences demonstrate a high homology with the neural cell adhesion molecule and other related cell surface glycoproteins of the immunoglobulin superfamily (6). The *DCC* gene showed allelic deletion in human colorectal, gastric, and esophageal carcinomas (6–8) and decrease or loss of expression in human colorectal and pancreatic carcinomas (6, 9). Down-regulation of *DCC* expression by antisense RNA transforms rat-1 fibroblasts (10). Prostate cancer is the most commonly diagnosed cancer in males in the United States and is the second leading cause of cancer death in men (11). With increasing prostate cancer incidence and an aging population, the mortality rate will increase again by 50% in the next 15 years. Risk factors for prostate cancer include dietary fat, androgens, and unknown genetic and environmental factors (11). Thus far, a few studies regarding tumor suppressor genes were carried out in prostate cancer. It was reported that *RB* mRNA was truncated in human DU 145 prostatic carcinoma cells. Transfection with a normal *RB* gene suppressed cell tumorigenicity in nude mice (12). Isaacs *et al.* (13) showed that prostatic carcinoma cell lines (TSUPr-1, PC-3, and DU 145) contain mutations in the *p53* gene and that transfection of the wild-type *p53* gene reduces their tumorigenicity. In the present study, we have assessed the *DCC* gene in prostatic carcinoma at both mRNA expression and LOH levels. *DCC* mRNA expression was measured in 4 human and 5 rat prostatic carcinoma cell lines as well as 14 patient tissue samples. Loss of heterozygosity of the *DCC* gene was determined in human carcinomas. All of 9 carcinoma lines (100%) and 12 of 14 tumor tissues (86%) showed a decrease in *DCC* expression. Five of 11 informative cases (45%) showed loss of heterozygosity at the *DCC* locus. Our data demonstrate that loss of expression and allelic deletion of the *DCC* gene are frequently observed in prostatic carcinoma cells.

Materials and Methods

Tumor Cell Lines. Human prostatic carcinoma cell line DU 145, PC-3, and LNCaP were obtained from American Type Culture Collection (Rockville, MD). PPC-1 and rat prostatic carcinoma cell lines AT-2.1, AT-3, MAT-Lu, MAT-LyLu and GP9F3 were kindly provided by Drs. S. R. Wolman and K. J. Pienta (Wayne State University), respectively. All cells were grown in RPMI 1640 supplemented with 10% fetal calf serum.

Tissue Specimens. Matching prostatic carcinoma and normal tissues were obtained from 14 patients who underwent radical prostatectomy. Tissue samples were immediately frozen at −80°C. A 6-μm section was cut from each tissue and stained with hematoxylin/eosin. Samples containing no carcinoma cells were considered normal and samples containing more than 70% carcinoma cells were considered tumor.

Total RNA Isolation and DNA Extraction. Total RNA from cell lines and tissues was isolated by the guanidinium isothiocyanate/CsCl method (14). Tissues were ground to powder in liquid nitrogen. DNA from cell lines and tissue powder was extracted as previously described (14).

Reverse Transcription-Polymerase Chain Reaction. RT-PCR was performed as described previously (14) with some modifications. *DCC* complementary DNA was amplified at 94°C for 1 min; 56°C for 2 min, and 72°C for 1.5 min for 35 cycles in the GeneAmp PCR system 9600 (Perkin Elmer Cetus). *DCC* primers are located on exons O and P and amplify a 233-base pair fragment from both human and rat mRNA (6). A fragment of this size cannot be amplified from genomic DNA, since the primers were designed to frame sequences that cross an intron on the *DCC* gene (6). RT-PCR of GAPDH was used as quantitative control. GAPDH primers are suitable for human and rat and give a 598-base pair amplified fragment (15). Samples were run at 94°C for 1 min; 49°C (in human) or 47°C (in rat) for 2 min, and 72°C for 1.5 min for 35 cycles. RT-PCR without RNA or without reverse transcriptase were included in each experiment as negative controls. Primers used were 5′-TTCCGCCATGGTTTTTAAATCA-3′ (*DCC* sense), 5′-AGCCTCATTTTC-AGCCACACA-3′ (*DCC* antisense), 5′-CCACCCATGGCAAATTCCATG-GCA-3′ (GAPDH sense), and 5′-TCTAGACGGCAGGTCAGGTCCAC-3′ (GAPDH antisense).

PCR-LOH Analysis. Fifty to 500 ng of genomic DNA were held at 85°C for 5 min in 100 μl buffer containing 10 mM Tris (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.1 μg/μl bovine serum albumin, 1 μM concentrations of sense and antisense primers. Then 2.5 units of AmpliTaq DNA polymerase (Perkin Elmer Cetus) was added and PCR was run at 95°C for 1 min; 57°C for 30 s, and 72°C

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[3] The abbreviations used are: *DCC*, deleted in colon carcinoma; LOH, loss of heterozygosity; PCR, polymerase chain reaction; RT, reverse transcription; VNTR, variable number of tandem repeats; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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for 30 s for 35 cycles. For M2 and M3 polymorphism (6, 16), PCR products were digested with *Msp*I and analyzed on 2.5% Metaphor agarose gels (FMC Bioproducts). For VNTR polymorphism (8), PCR products were directly separated on 2.5% gels. The primers were: 5'-GATGACATTTTCCCTCTAG-3' (VNTR set 1 sense), 5'-GTGGTTATTGCCTTGAAAAG-3' (VNTR set 1 antisense); 5'-TCCCTCTAGAAATTGTGTG-3' (VNTR set 2 sense), 5'-T-GACTTTATCTCATTGGAG-3' (VNTR set 2 antisense); 5'-TGCACCAT-GCTGAAGATTGT-3' (M2 sense), 5'-AGTACAACACAAGGTATGTG-3' (M2 antisense); 5'-CGACTCGATCCTACAAAATC-3' (M3 sense), and 5'-TCTACCCAGGTCTCAGAG-3' (M3 antisense). Negative controls without genomic DNA were performed for each set of PCR reaction.

Gel Densitometry Scanning. The intensity of individual bands was measured by densitometry scanning using an LKB 2400 GelScan XL laser densitometer (Pharmacia).

Data Analysis. *DCC* mRNA expression was calculated by normalization of the amount of the 233-base pair *DCC* fragment to the 598-base pair GAPDH fragment. LOH was defined by a visible change in that allele:allele ratio in tumor compared to matching normal tissue. In human prostatic carcinoma cell lines, comparison was made with normal control prostate tissue DNA. Allelic deletion of *DCC* was judged by a positive LOH at any of the three sites M2, M3, and VNTR.

Results

***DCC* mRNA Expression.** *DCC* expression was examined in 4 human and 5 rat prostatic carcinoma cell lines as well as in 14 matched normal and carcinoma tissues by semiquantitative RT-PCR. In human carcinoma cell lines, DU 145 and PPC-1 transcribed *DCC* mRNA at a level of approximately 40% of the average *DCC* expression in normal human prostate tissues. *DCC* expression in PC-3 and LNCaP cells was undetectable (Fig. 1*a*; Table 1). Among 5 rat Dunning prostatic carcinoma cell lines, AT-2.1 and GP9F3 cells expressed *DCC* at about 10 to 15% of the level of that in normal rat prostate tissue. AT-3, MAT-Lu, and MAT-LuLy cells had completely lost its expression (Fig. 1*b*; Table 1). In patient tissues, 3 carcinoma samples showed complete extinction of *DCC* expression, and 9 samples showed different degrees of reduction compared to matching normal tissues (Fig. 1*c*; Table 1). In two cases, tumor tissues expressed levels of *DCC* that were comparable to or higher than their normal counterparts (Fig. 2; Table 1). In this study, we have observed large differences in *DCC* expression among different individuals. For instance, patient 12 expressed approximately 10 times more *DCC*

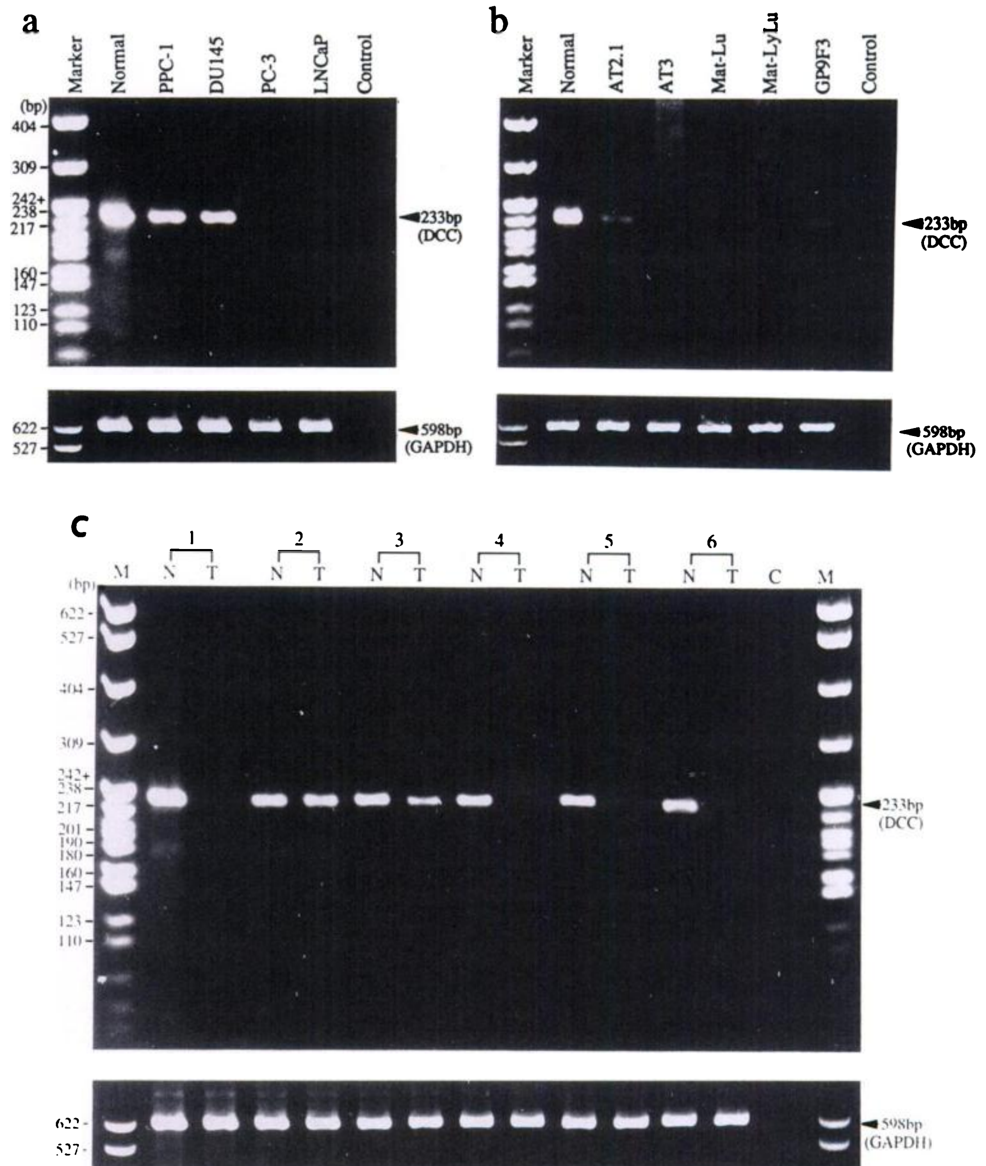

Fig. 1. *DCC* expression in prostatic carcinomas. *DCC* complementary DNA was amplified by PCR and analyzed on 2.5% agarose gels. GAPDH was used as quantitative control. (*a*) *DCC* expression in human prostatic carcinoma cell lines. *Marker*, PBR322/*Msp*I; *Normal*, normal human prostate tissue; *Control*, PCR sample without RNA. (*b*) *DCC* expression in rat Dunning prostatic carcinoma cells. *Marker*: PBR322/*Msp*I; *Normal*, normal rat prostate tissue; *Control*, PCR sample without RNA. (*c*) *DCC* expression in patient tissues. *M*, PBR322/*Msp*I; *N*, normal; *T*, tumor; *1*, patient 14; *2*, patient 10; *3*, patient 13; *4*, patient 5; *5*, patient 3; *6*, patient 6. *bp*, base pairs.

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Table 1 *Decreased expression and LOH of DCC gene in prostatic carcinomas*

Origin	Name	Tissue	*DCC* expression (% of normal)	*DCC* polymorphic sites		
				M2	M3	VNTR
Human tumor lines		N(h)[a]	100[b]	He	He	He
	PPC-1		43	Ho(c)	He	Ho
	DU 145		40	Ho(c)	He	Ho
	PC-3		0	Ho(n)	Ho(c)	Ho
	LNCaP		0	Ho(n)	Ho(c)	Ho
Rat tumor lines		N(r)	100[b]			
	AT-2.1		11			
	GP9F3		14			
	AT-3		0			
	MAT-LuLy		0			
	MAT-Lu		0			
		N(h)	100[c]	He	He	Ho
Patients	1	T(h)	56	Ho(n)	Ho(c)	Ho
		N(h)	100	Ho(n)	Ho(c)	Ho
	2	T(h)	72	Ho(n)	Ho(c)	Ho
		N(h)	100	He	He	He
	3	T(h)	16	He	He	He
		N(h)	100	He	Ho(n)	He
	4	T(h)	69	He	Ho(n)	Ho
		N(h)	100	He	Ho(n)	Ho
	5	T(h)	0	He	Ho(n)	Ho
		N(h)	100	He	He	He
	6	T(h)	0	Ho(c)	Ho(n)	Ho
		N(h)	100	Ho(c)	Ho(n)	Ho
	7	T(h)	59	Ho(c)	Ho(n)	Ho
		N(h)	100	He	He	Ho
	8	T(h)	115	He	He	Ho
		N(h)	100	Ho(n)	Ho(c)	He
	9	T(h)	24	Ho(n)	Ho(c)	Ho
		N(h)	100	Ho(c)	Ho(c)	He
	10	T(h)	101	Ho(c)	Ho(c)	He
		N(h)	100	Ho(c)	Ho(n)	Ho
	11	T(h)	8	Ho(c)	Ho(n)	Ho
		N(h)	100	Ho(c)	He	He
	12	T(h)	3	Ho(c)	He	Ho
		N(h)	100	Ho(c)	He	He
	13	T(h)	50	Ho(c)	He	He
		N(h)	100	Ho(c)	Ho(n)	He
	14	T(h)	0	Ho(c)	Ho(n)	He

[a] N(h), normal human prostate tissue; N(r), normal rat prostate tissue; T(h), human prostatic carcinoma; He, heterozygous; Ho(c), homozygous, cut by *MspI*; Ho(n), homozygous, not cut by *MspI*.
[b] Average of *DCC* expression in normal human or rat prostate tissues was considered as 100%.
[c] *DCC* expression in matched normal human prostate tissue was considered as 100%.

mRNA than patient 1 (Fig. 2). However, in a majority of cases tumor tissues expressed less *DCC* than their normal counterparts. On average, tumor tissues expressed *DCC* mRNA at about a 30% level compared to that in normal tissues (Fig. 2). Altogether, 9 of 9 (100%) prostatic carcinoma cell lines and 12 of 14 (86%) patient samples showed a reduction or loss of *DCC* expression.

Loss of Heterozygosity. Allelic deletion of the *DCC* gene was determined by PCR-LOH in human carcinomas. In order to increase assay sensitivity, three different sites, *i.e.*, M2, M3, and VNTR were used in this study. A positive allelic deletion of *DCC* was judged by LOH at one or any combination of these three sites (Fig. 3). We found that it was heterozygous at the M3 site and homozygous at the M2 and VNTR sites in PPC-1 and DU 145 cells and homozygous at all three sites in PC-3 and LNCaP cells (Table 1). Compared to normal control prostatic tissue, we considered that all 4 human cell lines had allelic deletion at the *DCC* locus. In patient samples, 11 of 14 cases were informative and 5 of them (45%) had allelic deletion (Table 1).

Discussion

The putative tumor suppressor gene *DCC* was originally identified in colon carcinomas by virtue of its high frequency of deletion in this cancer. The gene has been partially cloned and mapped to chromosome 18q21.3 (6). Recent evidence indicates that the *DCC* gene is involved not only in colorectal carcinoma but also possibly in pancreatic, gastric, and esophageal carcinomas (6–9). Introduction of chromosome 18 into a human colon carcinoma cell line suppressed its tumorigenicity (17). Down-regulation of *DCC* expression by antisense RNA leads to transformation of rat-1 fibroblast cells (10). In the present study, we have explored a possible involvement of the *DCC* in prostate cancer. Our results demonstrate, for the first time, that loss of

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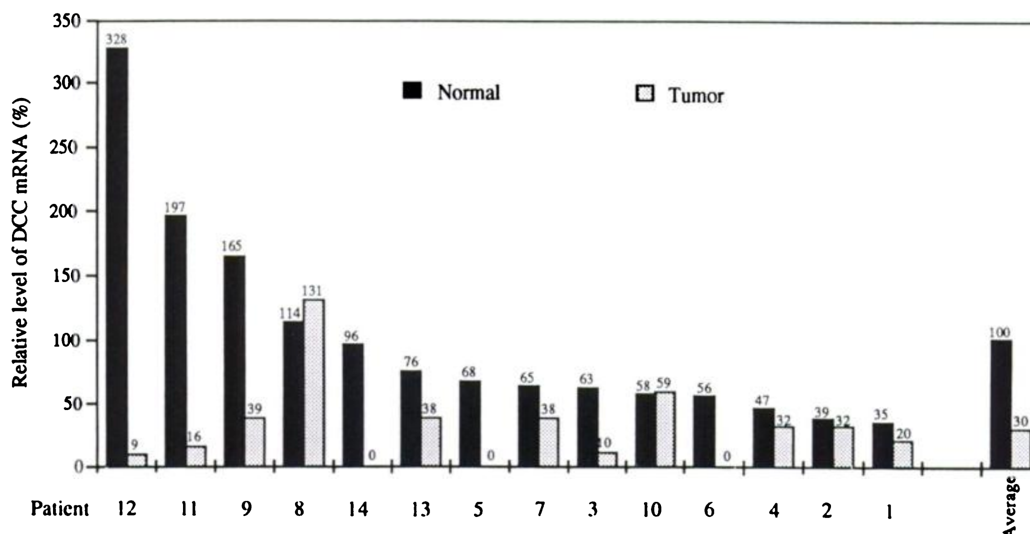

Fig. 2. Comparison of *DCC* expression in patient tissues. Average of *DCC* expression in 14 normal tissues was calculated and considered as 100%. Shown is the relative expression to the normal average for both normal and tumor samples.

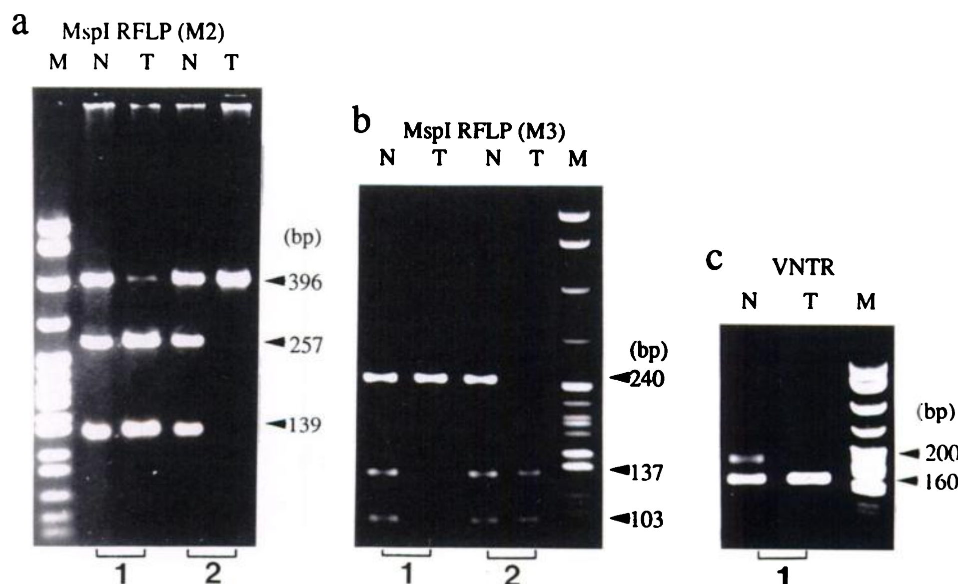

Fig. 3. PCR-LOH of *DCC* gene in prostatic carcinomas. Representative cases of LOH at M2 (*a*), M3 (*b*) and VNTR (*c*) are shown. *M*, PBR322/*MspI*; *N*, normal; *T*, tumor; *1*, patient 6; *2*, patient 1.

expression is frequently observed in prostatic carcinomas. We have also shown a high frequency of allelic deletion at the *DCC* locus in prostatic carcinoma cells. These data suggest that inactivation of the *DCC* gene may play an important role in the development of prostate cancer, the most common cancer in men. Previously, Carter *et al.* (18) have reported that the frequency of LOH at *DCC* locus in 28 prostate cancer patients was around 17%. The difference in frequency between their (17%) and our study (45%) could be due the methods used to detect LOH. They have determined M2 and M3 site LOH by Southern blot analysis, whereas we have used three sites for LOH detection which increases assay sensitivity. In fact, the M2 plus M3 site LOHs were 18% in our study.

The *DCC* gene encodes a molecule which shares high homology with the neural cell adhesion molecule (6). Cell adhesion molecules are cell surface receptors which play critical roles during processes such as embryogenesis, thrombosis, wound healing, cell homing, immunoreaction, as well as tumor progression and metastasis. It has been shown that E-cadherin, a member of the cell adhesion molecule family, has the ability to suppress cell invasion (19). Intriguingly, in this study *DCC* expression seems to be inversely correlated with the metastatic potential of rat Dunning prostate carcinoma cells. Highly metastatic cell lines, *i.e.*, AT-3, MAT-Lu, and MAT-LuLy cells (20), have completely lost *DCC* expression, whereas low metastatic cells AT-2.1 and GP9F3 (20) express *DCC* at levels approximately 10 to 15% of those in normal rat prostate tissue. A similar observation was made in human colon cancer where *DCC* expression was reduced during tumor progression from intramucosal to invasive carcinoma (21). This raises the possibility that the *DCC* may act as a metastatic suppressor.

Results from the present study indicate that the frequency of reduction in *DCC* expression is 2 times higher than the frequency of LOH at the *DCC* locus in prostatic carcinoma cells. It will be interesting to determine mechanism(s) of reduced *DCC* expression in prostate cancer as well as other cancerous diseases.

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