Short Communication

Myopodin, a Synaptopodin Homologue, Is Frequently Deleted in Invasive Prostate Cancers

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Prostate cancer is one of the leading causes of cancer-related deaths for men in the United States. Like other malignancies, prostate cancer is underscored by a variety of aberrant genetic alterations during its development. Although loss of heterozygosity or allelic loss is frequently identified among prostate cancers, few genes have been identified thus far as critical to the development of invasive prostate cancers. In this report, we used the recently developed technology, the “differential subtraction chain,” to perform a genome-wide search for sequences that are deleted in an aggressive prostate cancer. Among the deleted sequences, we found that one sequence was deleted in >50% of prostate cancers we tested. We mapped this sequence to chromosome 4q25 by screening the Genebridge 4 hamster radiation panel with primers specific to this probe, and subsequently identify a 54-kb minimal common deletion region that contains the sequence encoding myopodin. Sequence analysis indicates that myopodin shares significant homology with synaptopodin, a protein closely associated with podocyte and neuron differentiation. Further study shows that frequent complete or partial deletions of the myopodin gene occurred among invasive prostate cancer cases (25 of 31 cases, or 80%). Statistical analysis indicates that deletion of myopodin is highly correlated with the invasiveness of prostate cancers, and thus may hold promise as an important prognostic marker for prostate cancers. (*Am J Pathol* 2001, 159:1603–1612)

Prostate cancer remains one of the most frequently diagnosed malignancies in American men. Approximately 37,000 men die from this disease annually.¹ Despite the recent advances in our understanding of the environmental, hormonal, and nutritional parameters affecting the incidence of prostate cancers, much remains to be learned about the pathogenesis of prostate cancer. Epidemiological and laboratory studies indicate that genetic factors are important in the pathogenesis of prostate cancers.^{2,3} For example, 9% of prostate cancer cases have a strong familial component that is inherited as an autosomal dominant trait with high penetrance. In addition, cytogenetic studies, fluorescent *in situ* hybridization, comparative genomic hybridization, and allelotype analyses have revealed numerous genetic abnormalities associated with invasive prostatic carcinoma, including loss or gain of regions of several chromosomes, presence of trisomies, amplification of certain genes in X chromosome, loss of Y chromosome, and high frequency of loss of heterozygosity in several hot spots. These aberrant genomic alterations seem to accumulate with advancing stages of prostate cancers. However, it is not clear what molecular events are responsible for the progression of prostate cancer from a relatively indolent disease to one that could be life threatening.

In this report, we applied a methodology previously developed from this laboratory, namely, the “differential subtraction chain” (DSC) to identify sequences that were deleted in prostate cancer genome. One of the deleted sequences identified in an aggressive prostate cancer was found similarly deleted in many other prostate cancer genomes. We mapped out a common deletion region among the prostate cancers, and identified a gene named “myopodin” that was within the minimal common deletion region. Further study indicated that there were

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widespread complete or partial deletions of the myopodin gene in invasive type of prostate cancers.

Materials and Methods

Preparation of Tissue Material

Protocols for tissue procurement, specimen storage, and informed consent were established in the Western Pennsylvania Tissue Bank, and approved by the Institutional Review Board. Freshly frozen, macrodissected tissue was used for DSC reaction. Samples of prostate cancers were dissected and trimmed to obtain pure tumor (completely free of normal prostate acinar cells). Sandwich frozen sections were performed to examine the purity of the tumors. These tissues were then homogenized. Matched frozen blood sample from the same individual was used for preparing tester amplicons. Microdissected tumor cells were used in subsequent validation analysis and survey studies. Five- μm thin sections of selected prostate cancer frozen tissues were stained with hematoxylin and eosin and visualized under the microscope. Laser capture microdissection was performed to obtain pure tumor samples. The genomic DNA was extracted and purified. The integrity of the DNA was examined by polymerase chain reaction (PCR) with a set of primers located away from chromosome 4. These DNA samples were then screened for genomic deletion. Genomic DNA from tissue or blood samples was extracted as previously described.⁴ Clinical follow-up information of the specimen was obtained from the database of Western Pennsylvania Tissue Bank. All information in the database was coded so that the patient's identification was protected. Investigator had no access to the patient's geographical or personal information.

Genomic Amplicon Generation

Procedure for genomic amplicon generation was described previously.⁴ One μg of genomic DNA from the tumor or the blood cells of the same individual was digested with 20 U of *EcoRI* at 37°C for 5 hours. The digestion mixture was purified by QiaQuick PCR purification kit (Qiagen, Valencia, CA), and ligated with adapter sequences *Eco*IIa/IIb (tumor sample, ACCGTATCCAAG-GTCACGTACGAG/pAATTCTCGTACG) or *Eco*IIa/IIb (blood sample, GTCCAAGCAGTTCATAGTCAGCCG/pAATTCGGCTGAC) by T4 DNA ligase (New England Biolabs, Beverly, MA) at 25°C for 6 to 16 hours. The ligation mixture was purified, and amplified by PCR (94°C for 1 minute, then 94°C for 30 seconds, 68°C for 3 minutes for 30 cycles).

DSC

One μg of blood amplicons (testers) was mixed with 10 μg of tumor amplicons (drivers), which had been digested with *EcoRI* to remove the attached adapter sequences, in a total volume of 7 μl . One μl of 30 $\times$ EE buffer (300 mmol/L 2-hydroxyethylpiperazine-N'-3-pro-

panesulfonic acid (EPPS) and 30 mmol/L ethylenediaminetetraacetic acid, pH 8.0) was added. The mixture was then heated to 98°C for 3 minutes, and 2 μl of 5 mol/L NaCl were added to the reaction to give the final concentration of 1 mol/L while maintaining the temperature at 98°C. The mixture was then incubated at 98°C for an additional 2 minutes and hybridized at 67°C for 20 hours. The hybridization mixture was purified by sodium acetate/ethanol precipitation. The pellet was then washed with 70% ethanol. The dry pellet of the hybridization mixture was reconstituted in 50 μl of 1 $\times$ Mung bean nuclease buffer and digested with 10 U of Mung bean nuclease at 30°C for 30 minutes. The digestion products were treated with 0.5 μl of 10% sodium dodecyl sulfate and purified with ethanol purification. The DNA was resuspended in 9 μl of 3 $\times$ EE buffer. One μl was removed for PCR (quality control). The remainder of the sample was reheated to 98°C for rehybridization. This procedure was repeated twice.

DSC Product Cloning, Colony Screening, and Sequencing

Round 2 and 3 DSC products were amplified by PCR (94°C for 1 minute, then 30 cycles of 94°C for 30 seconds, 68°C for 3 minutes). The amplified products were visualized by agarose gel electrophoresis. An aliquot of the amplified products was ligated with TOPO TA cloning vector (Invitrogen, Carlsbad, CA) and transfected into *Escherichia coli*. Three hundred four colonies were randomly picked and grown overnight in LB broth with 100 $\mu\text{g}/\text{ml}$ of ampicillin. Aliquots of the bacterial culture were spotted onto colony screen filter and hybridized with amplicons generated from blood or tumor prostate tissues. To identify which DSC fragment was not present in the tumor sample, only those colonies without hybridization signal for tumor amplicons but positive for blood amplicons were selected. DNA was extracted from the selected colonies and sequenced with M13 forward or reverse primers using the thermocycle sequenase dideoxynucleotide termination method (United States Biochemicals, Cleveland, OH).

Northern Blot Analysis

We analyzed myopodin expression by Northern blot analysis of 20 μg total RNA as described.⁵ The myopodin gene probe was generated from a PCR product encompassing exon 2 of myopodin using primers E1/E4 (GCAT-TGCCCT TCTTCTAACGGA/ATAGCAGATTGACAGTGACAGC).

PCR Mapping and Survey

The information of the primers specific for STS in chromosome 4q are listed in Figure 2. The PCR conditions and the physical map of these STS were obtained through the website of Stanford Genome Center (<http://www-shgc.stanford.edu>). The primers used for screening

Table 1. Primer Sequences Specific for Myopodin Exons

Name of primers	Sequence pairs
E35/E36	cacgaggagtgagagtgtac/acactccttctgcctgaa
E37/E38	aggagtggtggattctcca/ctgtgaggtctgctgtgat
E1/E2	gcattcgcccttcttaacgga/ctgtcaccttctctcctcgt
E39/E40	gaaggtagacaaggaggatac/catctgtcccctctgttcag
E41/E42	ggggacaagatggagatgtt/tctgggcagcatctgttct
E43/E44	gagaaccacgacttcttacc/tcctctggatgttgctgtc
E45/E46	tggccccatgaatagaacg/acttgaacccctctggaga
E47/E48	ggtttcaagtccgattgctg/ttgctggcactgagatctt
E49/E50	aggatctcagtgccagcaaa/gtgccccgttgccttctgaatt
7A/7B	agaagcgaaacggggcac/gggaggatgaggacttgac
E5/E6	cccaaccagtaactccagt/ggtgtgtaattctgactggc
E7/E8	caccacaaccacaggttcc/cgtgtctgaatcgaccacat
E9/E10	atgtggtcgattcagacacg/atggctgagactgagagca
E11/E12	tgcttcaagtctcagccat/catccttgcacatgaggt
9A/9B	cctgttacttccaacctcc/cgtaggtaggacagcattc
E13/E14	atgctgcctcacctacgaat/ctgattctgtttggcgag
E15/E16	ctcgccaaagcaagaatcag/agcaaatggacactcagct
E17/E18	tccctgagaatgcctagcaa/tttggggtgctcctggatt
E19/E20	ggagcaccacaaaatagaca/gaaccttaccataggagg
E21/E22	cctctatggtgaaagggtt/aacagggtggctgagattgac
E23/E24	cagagaagatactcggagga/aggattccaggcatgaacca
E25/E26	ctggaatcctagcacttgg/gagggtgggaatcaacctt
E27/E28	cctccccagttacttaagga/gtggtttctccaggtgaaac
E29/E30	gaaccacatgattgggacc/gaacgtgggagaagcagttg
E31/E32	tcccacgttccttgcattc/ttcaactcatagccagctgg
E33/E34	ccagctggctatgaatgaa/gatgacttttctggagctg
E3/E4	cagctccaagaaaagtcattac/atagcagattgacagtgcagacg

partial deletion of myopodin were listed in Table 1. The following PCR condition was used: 94°C for 1 minute, then 30 cycles of 94°C for 30 seconds, 59°C for 1 minute, and 72°C for 2 minutes. Twenty-five to 50 ng of DNA templates were used in each reaction. Genebridge 4 hamster radiation hybrid panels were purchased from Research Genetics, Huntsville, AL. PCR using primers specific to 12C was performed on the panel DNA. The results were tabulated as positive or negative for each reaction, and input into Genebridge 4 RH program of <http://www-genome.wi.mit.edu>.

In Situ Hybridization

5'-digoxigenin-labeled 24-mer oligonucleotides corresponding to antisense or sense sequence (control) of myopodin were incubated with pronase digested, prehybridized, 3- μ m thin sections of prostate tissue from paraffin-embedded tissue blocks at 37°C for 12 hours in hybridization solution.⁶ The slides were washed twice with 2 $\times$ standard saline citrate for 15 minutes, and then twice with 1 $\times$ standard saline citrate for 15 minutes. The slides were then incubated with horseradish peroxidase-conjugated anti-digoxigenin antibody for 1 hour at 37°C. Chromogenic color was developed using the peroxidase substrate diaminobenzidine. The sense probes were used as a negative control.

Statistics

A permutation test⁷ with general scores was used to detect a difference in binomial response rates with in-

creasing invasiveness. Equally weighted scores (equivalent to the exact Mantel-Cox test⁸) were used to test for trend.

Results and Discussion

Previously we developed the DSC, a methodology to identify the sequences of the differences between two DNA samples.⁴ This method enriches unique tester sequences double-exponentially, and efficiently isolates small representative sequences of large differences. We hypothesize that prostate cancer cells contain deletions that harbor genes important in cancer development. To initiate the study, we chose to perform DSC on a case of aggressive prostate cancer to examine the DNA sequences that were deleted from the genome. We generated mixtures of DSC subtraction products using *Bam*HI and *Eco*RI genomic amplicons derived from the prostate cancer (drivers) to subtract the counterparts from blood cells (testers) of the same patients (Figure 1). These products were PCR-amplified, ligated into TA cloning vector, and subsequently transfected into competent *E. coli*. To select DSC products that represent deletions in the cancer genome, 304 colonies were picked and screened by hybridization with amplicon probes derived from cancer or blood cells. Colonies were selected when they hybridized with amplicons from blood cells but were negative with cancer. We sequenced DNA from the colonies of interest, and synthesized primers corresponding to these sequences. PCR was performed on the genomic DNA templates to determine the presence of deletions. Although many of these probes represent loss of heterozygosity or loss of alleles, seven were found to represent the regions of homozygous deletion (Figure 1C).

To evaluate the frequency of genomic deletion in these regions among prostate cancers, we used six pairs of primers specific to these sequences to screen a panel of 18 cases of prostate cancer tissues and cell lines. More than 50% (10 of 19, including the case used for DSC) of the cases contained deletion in a specific (12C) sequence (Figure 2). Screening with other DSC probes representing homozygous deletions of the initial case on the same panel, however, revealed no additional deletion (data not shown), suggesting that these homozygous deletions were only specific to the initial case. These results probably reflect the heterogeneity of prostate cancers.

To determine the chromosomal location of 12C, we screened the Genebridge 4 hamster radiation hybrid panel using primers specific for 12C, and located this probe to the locus of 4q25-26. Because 12C is a small sequence that represents larger deletions in the genomes, deletion mapping by PCR using a panel of primers specific for markers (25 pairs) adjacent to 12C were performed on the templates of the panel cases. We identified various sizes of deletions, ranging from 500 kbp to >23 mega-bp among the analyzed cases, and mapped a minimum common deletion region of at least 54 kb (Figure 2).

Figure 1. DSC enrichment of amplicons that are deleted in prostate cancer. **A:** Agarose electrophoresis of DSC products using blood amplicons (*EcoRI*) as testers and tumor amplicons (*EcoRI*) as drivers, after round 0 (lane 1), round 1 (lane 2), round 2 (lane 3), and round 3 (lane 4) of subtraction. **B:** Screening of DSC products from round 2 and round 3 DSC of **A** with ^{32}P -labeled tumor amplicons or blood amplicons. **C:** Electrophoresis of PCR products from selected primers using microdissected genome templates from tumor (lanes 2, 4, 6, 8, 10, 12, 14, and 16) and its matched blood cells (lanes 1, 3, 5, 7, 9, 11, 13, and 15) with primers 11D (lanes 1 and 2), 12D (lanes 3 and 4), 12C (lanes 5 and 6), 1E (lanes 7 and 8), 1F (lanes 9 and 10), 5F (lanes 11 and 12), 1G (lanes 13 and 14), and 12G (control primers, lanes 15 and 16).

To identify genes that might lie in the minimum common deletion region, we used marker sequences within the region to perform a homology search in the GenBank through the National Center for Biotechnology Information (NCBI) BLAST program. One marker generated a complete match with the myopodin coding sequence. The 4.2-kb mRNA transcript encodes a 698-amino acid protein with a predicted molecular weight of 83 kd (Figure 3A). The C-terminus of myopodin shares significant homology with synaptopodin (Figure 3B),^{9,10} a protein that is expressed in neurons and podocytes, and is involved in modulating actin-based shape and forming synaptic contact. Its expression is closely correlated with neuron or podocyte differentiation and stress.^{11,12} The N-terminus of myopodin contains a stretch of acidic amino acids, and shares homology with a group of nuclear localization proteins (Figure 3C).^{13–17} The physiological function of myopodin is not entirely clear. However, based on its homology to synaptopodin, one can hypothesize that the protein may play a role in maintaining cell shape and initiate cell-cell contact. Because synaptopodin is a marker of terminal differentiation, the expression of which is inactivated when cells proliferate,^{9,10} it is possible that the expression of myopodin is similarly related to differ-

entiation and plays an important role in maintaining cell shape that prevents mitosis.

Northern blot analysis indicated that myopodin was abundantly expressed in skeletal muscle, but also expressed in organs such as prostate, and small and large intestine (Figure 4A). *In situ* hybridization suggested that myopodin was primarily expressed in prostate epithelium with the strongest expression in the acinar cell layer (Figure 4B).

To determine whether partial deletions of myopodin gene are present in prostate cancers, a panel (39 cases) of DNA templates from microdissected prostate cancer samples was examined. PCR was performed with a set of primers overlapping the sequences for entire exon 1 and exon 2 of myopodin. More than 30% (12 of 39) of the cases were found to contain partial deletions in the exon 2 of myopodin. These deletions cover a range of sites within the gene. Most of the deletions (>90%), however, involve sequences that contain homology with synaptopodin (Figure 5). One case contained a small deletion in the 3'-untranslated region, whereas two contained deletion spanning the entire exon 2. Nine cases contain complete deletion of myopodin. Interestingly, no partial deletion was identified in exon 1, which contained the

Figure 2. Genome deletion analysis of prostate cancer. **A:** Survey of genomes of 18 primary prostate cancers and prostate cancer cell lines by PCR using a pair of primers specific to DSC probe 12C. Fifty ng of microdissected or cell line genomic DNA was used in the reaction. The experiments were repeated twice. A pair of primers away from chromosome 4q were used as control (4E AGTAGAGAGTGCTGGTCCACCTAG/AGGCATACTCTAGAAGACAGAGGC) (**bottom**). Primer sequences for 12C are GTATTCTAGCAAACCTGCTTAGCC/GGGCAGGGCAGTACCAAGGATGGC. **B:** Mapping the sizes of deletion in the genomes of prostate cancers. Chromosome 4 STS markers adjacent to DSC probe 12C were used as primers in PCR to map the sizes of deletions. Twenty-five to 100 ng of genomic DNA were used as templates in each reaction. DNA from the blood of the same patients and microdissected normal donor DNA were used as a control for each marker. The **green line** represents normal human genome. The **black lines** next to each case represent deletions. The line proportion of the map may not be interpreted as the exact size of physical deletion. Probe 12C was located between markers D4S832 and W1-11817.

mRNA start site and some of the untranslated leader sequence.

To characterize the prostate cancers that contain myopodin deletion, we categorized these cases by the invasiveness of the cancers, ie, 1) minimally invasive, by lack of evidence of capsular penetration or adjacent organ invasion, and by lack of disease progression and cancer-related deaths after radical prostatectomy; 2) invasive, by evidence of tumor penetration through the prostate capsule; 3) highly invasive, by evidence of tumor invading into seminal vesicles or adjacent organs; and 4) relapsing, by evidence of progression of the disease or deaths due to prostate cancer after radical prostatec-

tomy regardless of evidence of cancer invasiveness at the time of surgery. As demonstrated in Tables 2 and 3, the majority of the prostate cancers with myopodin deletion demonstrates a high level of invasion (>92% was at least invasive). Seven of nine relapsing cases contained partial or complete myopodin deletion. More than 84% of the cases with capsular penetration contained myopodin deletion. Interestingly, three cases were defined as organ-confined tumors at the time of radical prostatectomy, but relapse of cancer identified later. Myopodin deletions were identified in two of these three cases. Only 2 of 27 (7%) prostate cancers with myopodin deletion were identified as minimally invasive. An exact Mantel-Cox Test for

A

[illegible]**B**

Myopodin: 191 **NRTA** K **FF** 197// 281 **VPA** KR **TGIL** Q **E** AK- **RR** STTKP **MPTF** K **E** - **FKV** S **PNP** E **LL** S **L** L **Q** NSEG **KR** 328
Synaptopodin: 231 **NRTA** R **FF** 237// 359 **VPA** SK **TGIL** E **E** SMA **RR** GSRRS **MPTF** V **E** K **FKV** T **PNP** E **LL** D **L** L **Q** TADE **KR** 406

Myopodin: 334 S **G** P **EE** DYLS **LGAEA** C **NF** M **Q** 352// 474 **SG** R **GA** E **L** F A **K** **RQSRMEKYV** VD// 502 **AR** AQ **SPT** P **SLP** A **SWKY** SS **N** V
Synaptopodin: 414 V **G** V **EE** EPFA **LGAEA** S **NF** Q **Q** 432// 477 **SG** R **GA** E **L** Y A **K** **RQSRMEKYV** IE// 506 **AR** CP **SPT** M **SLP** S **SWKY** PT **N** A

Myopodin: 674 **APRP** K **FS** AKKS **G** VTI **QVWKPS** 694
synaptopodin: 661 **APRP** S **FS** TRNA **G** IEA **QVWKPS** 681

C

Myopodin:	25	E	A	DEEEE	GDK	E	DTCEVAFPLGAS	E	SEVD	E	LLSDVDNDTQVNVN	60
Eip78C:	64	E	E	DEEEE	GEE	E	E-----E	E	DE-D	E	ALLP-----VVNFN	90
Sialoprotein:	151	E	S	DEEEE	ESE	E	E-----E	E	NE-N	E	A-EVDENEQVNVN	181
C1P1-interacting zinc finger protein:	741	E	G	DEEEE	EDD	E	D-E-----E	E	IEVE	E	L 762	
Nuclear protein np94b:	660	E	G	DEEEE	EDD	E	D-E-----E	E	IEVE	E	L 681	
CI21 like protein:	680	E	G	DEEEE	EDD	E	D-E-----E	E	IEVE	E	L 701	
Ecdysone-inducible protein E78-A:	64	E	E	DEEEE	GEE	E	E-----DE	E	EDED	E	ALLP-----VVNFN	94

Figure 3. Sequence of myopodin gene and its homology with synaptopodin and other proteins. **A:** Nucleotide sequence and the predicted amino acid sequence of myopodin. The open reading frame of myopodin predicts a 698-amino acid protein. **B:** Amino acid sequence homology of myopodin with synaptopodin. Significant homology between myopodin and synaptopodin were found in six stretches of sequences of myopodin. **C:** Homology of myopodin sequence with several other proteins. An acidic amino acid-rich domain was found in the N-terminal sequence of myopodin, and it shares significant homology with several nuclear localization proteins.

Figure 4. Expression distribution of myopodin. **A:** Northern blot analysis of myopodin expression in 23 organ tissues. Twenty μ g of total RNA of pancreas (**lane 1**), kidney (**lane 2**), skeletal muscle (**lane 3**), liver (**lane 4**), lung (**lane 5**), placenta (**lane 6**), brain (**lane 7**), heart (**lane 8**), leukocytes (**lane 9**), testes (**lane 10**), colon (**lane 11**), ovary (**lane 12**), small intestine (**lane 13**), prostate (**lane 14**), thymus (**lane 15**), spleen (**lane 16**), stomach (**lane 17**), thyroid (**lane 18**), spinal cord (**lane 19**), lymph node (**lane 20**), trachea (**lane 21**), adrenal gland (**lane 22**), and bone marrow (**lane 23**) were electrophoresed, Northern transferred to nylon membranes, and hybridized with a probe derived from exon 2 of myopodin. β -actin was used as the positive controls. **B:** *In situ* hybridization of prostate tissues with myopodin gene. A case of prostate cancer with deletion of myopodin gene was hybridized with a cocktail of digoxigenin-labeled antisense oligonucleotides corresponding to exon 2 of myopodin. H&E stain (**left**) and *in situ* hybridization (**right**) of the sections were shown for normal (**top**) and carcinoma (**bottom**).

Figure 5. Partial deletion of myopodin gene in prostate cancers. A 2.4-kb intron separates exon 1 and exon 2 of myopodin. The exon/intron boundary for myopodin is identified by mapping the mRNA sequence of myopodin to human genome draft through NCBI's BLAST program. Nonshaded area represents noncoding region, and green-shaded area represents coding sequence. represents acidic amino acid domain. The **black stripes** represent sequences containing homology with synaptopodin. PCRs were performed on genomic DNA of a panel of 39 cases of prostate cancers using primers in Table 1 to determine the presence of partial deletion. Deletions were further confirmed by sequencing on the PCR products.

Table 2. Pathological Grading, Clinical Outcome, and Myopodin Deletion

Case no.	Clinical outcome	Gleason grading at the time of prostatectomy	Pathology stage	Myopodin status
143B5	Died after 2.5 years	3 + 3 = 6	pT3a	Partial exon 2 deletion
143C4	Died after 8 years	3 + 4 = 7	pT3b	Exon 2 deletion
143E4	Died after 7.5 years	3 + 4 = 7	pT3a	Partial exon 2 deletion
150J1	Died after 1 month	3 + 3 = 6	pT4	No deletion
150I2	Recurrent	3 + 3 = 6	pT2b	Partial exon 2 deletion
151D1	Recurrent	3 + 3 = 6	pT2b	Partial exon 2 deletion
7270	Recurrent	4 + 5 = 9	pT3b	Complete deletion
269T	Recurrent	3 + 5 = 8	pT2b	No deletion
3806	Recurrent	4 + 5 = 9	pT3b	Complete deletion
143A2	Disease-free after 10 years	3 + 3 = 6	pT3a	No deletion
144A4	Disease-free after 10 years	3 + 2 = 5	pT2b	No deletion
143B1	Disease-free after 10 years	3 + 5 = 8	pT3b	Partial exon 2 deletion
143B3	Disease-free after 10 years	2 + 3 = 5	pT2b	No deletion
143C2	Disease-free after 10 years	3 + 4 = 7	pT3a	Partial exon 2 deletion
143D1	Disease-free after 10 years	2 + 2 = 4	pT3a	No deletion
143D5	Disease-free after 10 years	4 + 3 = 7	pT2b	No deletion
143E2	Disease-free after 10 years	3 + 2 = 5	pT2a	No deletion
143F1	Disease-free after 10 years	2 + 5 = 7	pT3b	Exon 2 deletion
143F3	Disease-free after 10 years	3 + 3 = 6	pT2b	Partial exon 2 deletion
3616	Disease-free after 7 years	4 + 3 = 7	pT2b	No deletion
323T	Disease-free after 3 years	3 + 3 = 6	pT2b	No deletion
15679	Disease-free after 2 years	4 + 4 = 8	pT3a	Complete deletion
7129	Disease-free after 2 years	4 + 5 = 9	pT2b	Complete deletion
678	Disease-free after 2 years	4 + 5 = 9	pT3b	No deletion
150J5	Disease-free after 2 years	3 + 4 = 7	pT2b	No deletion
151A4	Disease-free after 2 years	4 + 3 = 7	pT2b	No deletion
151B3	Disease-free after 2 years	2 + 5 = 7	pT3b	No deletion
151C2	Disease-free after 2 years	3 + 3 = 6	pT2a	Partial exon 2 deletion
151D5	Disease-free after 2 years	3 + 4 = 7	pT3a	Partial exon 2 deletion
151E4	Disease-free after 2 years	3 + 5 = 8	pT3a	Partial exon 2 deletion
151F3	Disease-free after 2 years	3 + 4 = 7	pT2b	No deletion
151G2	Disease-free after 2 years	3 + 4 = 7	pT2b	No deletion
314T	ND	3 + 3 = 6	pT3a	Complete deletion
318T	ND	3 + 4 = 7	pT2a	No deletion
13701	ND	4 + 4 = 8	pT3a	Complete deletion
6112	ND	3 + 5 = 8	pT3a	Complete deletion
9419	ND	3 + 3 = 6	pT3a	Complete deletion
10026	ND	5 + 4 = 9	pT3b	Complete deletion
17836	ND	3 + 3 = 6	pT3a	Complete deletion

ND, Not determined.

Table 3. Correlation of Myopodin Deletion or Gleason Score with Invasiveness of Prostate Cancers

Myopodin or Gleason	Minimal invasive (organ confined)	Invasive (capsular penetration)	Highly invasive (involving seminal vesicle)	Relapsing (progress after RP or cancer death)
Presence of myopodin deletion	14% (2/14)	84% (11/13)	78% (7/9)	78% (7/9)
Absence of myopodin deletion	86% (12/14)	16% (2/13)	22% (2/9)	22% (2/9)
Gleason ≥ 7 (poorly differentiated)	57% (8/14)	46% (6/13)	88% (8/9)	56% (5/9)
Gleason ≤ 6 (well to moderately differentiated)	43% (6/14)	54% (7/13)	12% (1/9)	44% (4/9)

RP, radical prostatectomy.

trend was tested for myopodin deletion association with invasiveness and was found significant ($P = 0.0002$). The myopodin deletion rates were similar among invasive, highly invasive, and relapsing cases and higher than for minimally invasive cancers. A permutation test with general scores (1-3-3-3 for minimally invasive, invasive, highly invasive, and relapsed, respectively) was significant ($P < 0.0001$) indicating a significant increase in myopodin gene deletion among cases with extracapsular penetration or relapse. In contrast, Gleason grading produced a far less clear conclusion, eg, 57% of the minimally invasive prostate cancer was graded as poorly differentiated based on the Gleason grading (≥ 7), and 54% of the invasive tumor was graded as well to moderately differentiated carcinoma (Gleason ≤ 6). The poorly differentiated tumors represented only 56% (5 of 9) of the relapsing cases. Approximately 66% of the well-differentiated to moderately differentiated cancers were classified as invasive or higher compared to 70% of the poorly differentiated cases. A Gleason score cut-off of 7 or higher was also tested for Mantel-Cox test for association with invasiveness, and found to be independent of degree of invasiveness (Mantel-Cox, $P = 0.5909$). Nevertheless, invasion of seminal vesicles by prostate cancers was closely correlated with high Gleason grades ($>88\%$).

Two recent comparative genomic hybridization studies indicated that up to 18% of sporadic prostate cancers contain abnormalities or deletions in the chromosome 4q,^{18,19} representing one of the most frequent deletion sites and suggested the presence of a tumor suppressor gene in the locus. In this report, we have uncovered myopodin that is frequently deleted in aggressive prostate cancers, and interestingly, is similarly located in chromosome 4q. The homology of myopodin with synaptopodin implies that myopodin may be one of the terminal differentiation protein that interacts with actin and maintains cell morphology and cell-cell contact. Our study indicates that deletion of myopodin, either partial or complete, is closely correlated with the invasiveness of prostate cancers. The high rate of deletions of myopodin in invasive prostate cancers and myopodin's homology with synaptopodin suggest that myopodin may play a role in maintaining normal cell-cell contact under physiological conditions. Interestingly, deletion of myopodin seems to produce higher specific rates in predicting the clinical

outcome (relapse) or in reflecting the cancer behavior (pathology defined invasion) than the morphological grading system (Gleason grading) does. Thus, myopodin may hold promise as an important prognostic marker for prostate cancers.

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