American Journal of Pathology, Vol. 165, No. 4, October 2004

Tumorigenesis and Neoplastic Progression

Molecular Genetic Evidence for Different Clonal Origins of Epithelial and Stromal Components of Phyllodes Tumor of the Prostate

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Phyllodes tumor of the prostate is a rare neoplasm, composed of epithelium-lined cysts and channels embedded in a variably cellular stroma. The pathogenetic relationship of the epithelium and stroma is unknown and whether each is a clonal neoplastic element is uncertain. We studied the clonality of phyllodes tumors from six patients who underwent either enucleation or transurethral resection as their initial treatment. This was followed by total prostatectomy in three of the patients. Laser-assisted microdissection was performed to extract epithelial and stromal components of phyllodes tumor from formalin-fixed, paraffin-embedded tissue. Polymerase chain reaction was used to amplify genomic DNA at specific loci on chromosome 7q31 (D7S522), 8p21.3-q11.1 (D8S133, D8S137), 8p22 (D8S261), 10q23 (D10S168, D10S571), 17p13 (TP53), 16q23.2 (D16S507), 12q11–12 (D12S264), 17q (D17S855), 18p11.22-p11 (D18S53), and 22q11.2 (D22S264). In each tumor, stroma and epithelium were analyzed separately. Gel electrophoresis with autoradiography was used to detect loss of heterozygosity. All tumors showed allelic loss in one or more loci of both the epithelial and stromal components. The frequency of allelic loss in the epithelial component was 2 of 5 (40%) at D7S522, 2 of 6 (33%) at D8S133, 1 of 5 (20%) at D8S137, 3 of 6 (50%) at D8S261, 4 of 4 (100%) at D10S168, 4 of 6 (67%) at TP53, 2 of 6 (33%) at D10S571, 6 of 6 (100%) at D16S507, 1 of 5 (20%) at D12S264, 1 of 6 (17%) at D17S855, 2 of 6 (33%) at D18S53, and 2 of 5 (40%) at D22S264. The frequency of allelic loss in the stromal component was 2 of 5 (40%) at D7S522, 1 of 6 (17%) at D8S133, 2 of 5 (40%) at D8S137, 3 of 6 (50%) at D8S261, 1 of 4 (25%) at D10S168, 3 of 6 (50%) at TP53, 2 of 6 (33%) at D10S571, 3 of 6 (50%) at D16S507, 1 of 5 (20%) at D12S264, 0 of 6 (0%) at D17S855, 1 of 6 (17%) at D18S53, and 0 of 5 (0%) at D22S264. The pattern of allelic loss is significantly different in both stroma and epithelium statistically; completely concordant allelic loss patterns were not seen in any tumor examined. Our data demonstrate that both epithelial and stromal components of phyllodes tumor of the prostate are clonal, supporting the hypothesis that both elements are neoplastic. While both epithelium and stroma are clonal proliferations, they appear to have different clonal origins. ***(Am J Pathol 2004, 165:1395–1400)***

Phyllodes tumor of the prostate is a rare neoplasm with poorly understood pathogenesis. Histologically, it resembles phyllodes tumor of the breast with hyperplastic epithelium-lined cysts and channels embedded in a variably cellular stroma.[1,2] A variety of terms have been used to describe these lesions, including phyllodes type of atypical hyperplasia, cystosarcoma phyllodes, and prostatic cystic epithelial-stromal tumor.[1–4]

The malignant potential of this tumor is unclear and has resulted in confusion in terms of prognosis and treatment. Unquestionably, the potential for sarcomatous transformation, recurrence, and infiltrative growth exists, but the frequency of these changes and their prognostic significance is unclear.[3–7]

Little is known about the genetic abnormalities in this tumor. Oncogene activation and tumor suppressor gene inactivation are important mechanisms in the genesis, propagation, and spread of most cancers, but the role of these processes in phyllodes tumor has not been previously explored. It is well known that allelic loss is a common early genetic alteration during tumorigenesis.[8–11] Previously, loss of heterozygosity analysis has

Accepted for publication June 4, 2004.

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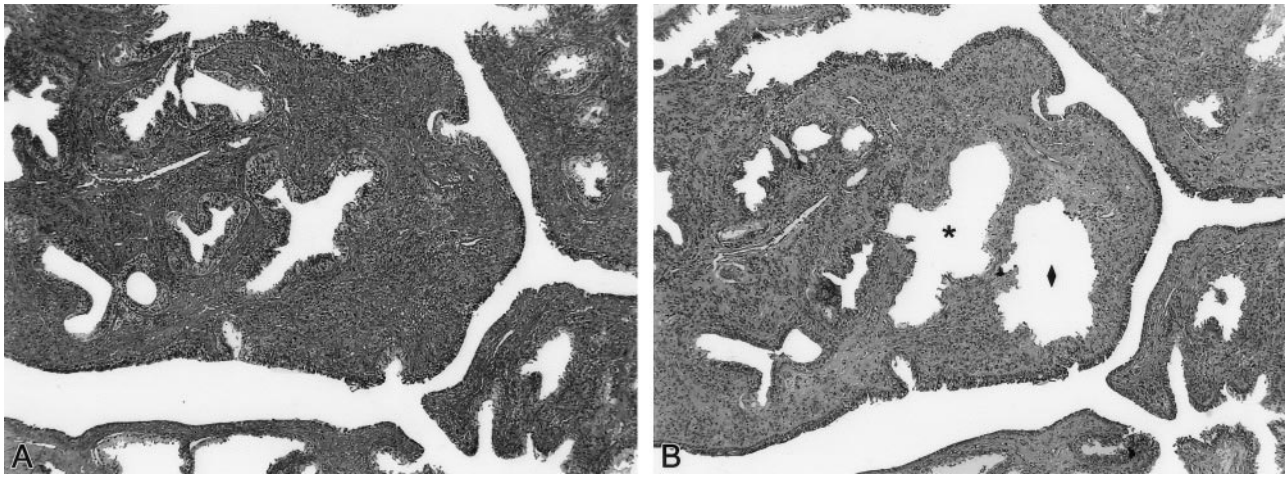

Figure 1. Laser microdissection of a phyllodes tumor of prostate (Case 3). It illustrated epithelial (*) and stromal (♦) components of tumor before microdissection (**A**) and after microdissection (**B**).

been used to study the clonal relationship of different components of the same tumor.[11–18] Since these genetic changes can be identified by allelic typing at polymorphic chromosomal loci, we compared the frequency of loss of heterozygosity and analyzed the pattern of allelic loss between the epithelial and stromal components.

Materials and Methods

Patients

Six men with phyllodes tumor of the prostate were included in our study. None of these cases were reported previously. Patients ranged in age from 25 to 88 years of age, with a mean age of 55, and all presented with urinary obstruction or painless hematuria. Four tumors were diagnosed by transurethral resection and two by retropubic prostatic enucleation. Three of the patients had no recurrence. The other three patients had multiple recurrences, first occurring at 1 and 6 months, and 10 years after diagnosis. Sarcoma emerged in one patient 11 years after the original diagnosis following three recurrences, while metastasis to the abdominal wall occurred in another patient. This research was approved by the Indiana University Institutional Review Board.

Tissue Samples and Microdissection

Histological sections were prepared from formalin-fixed, paraffin-embedded tissue and were stained with hematoxylin and eosin for microscopic evaluation. From these slides, the two components of the phyllodes tumors were identified (Figure 1). Laser-assisted microdissection of the two components was performed on the unstained sections using a PixCell II Laser Capture Microdissection (LCM) system (Arcturus Engineering, Mountain View, CA), as previously described.[12,19–22] Approximately 400 to 1000 cells of each component were microdissected from the 5-μm histological sections. Normal tissue (lymphoid tissue when present or normal epithelial cells) from each case was microdissected as a control.

We also performed experiments to compare allelic patterns from different parts of the same tumor. Different parts of the same component (epithelial or stromal) were sampled in three cases and left and right sides of the prostate, respectively, in one case (Case 6), in which prostatectomy had been performed.

Amplification of DNA

The dissected cells were de-paraffinized with xylene and ethyl alcohol. Polymerase chain reaction (PCR) was used to amplify genomic DNA at various specific loci on chromosome 7q31 (D7S522), 8p21.3-q11.1 (D8S133, D8S137), 8p22 (D8S261), 10q23 (D10S168, D10S571), 17p13 (TP53), 16q23.2 (D16S507), 12q11–12 (D12S264), 17q (D17S855), 18p11.22-p11 (D18S53), and 22q11.2 (D22S264). Previous studies demonstrated frequent loss of heterozygosity (LOH) on these chromosomes in prostatic intraepithielial neoplasia, prostatic carcinoma, and atypical adenomatous hyperplasia.[8,9,11,13,20,23] PCR amplification and gel electrophoresis were performed as previously described.[9,11–16,24] PCR for each polymorphic microsatellite marker was repeated at least twice from the same DNA preparations and the same results were obtained.

Analysis of Allelic Loss Pattern

When the genetic material in a patient was found to be homozygous for the polymorphic markers (ie, showing only one allele in the normal control tissue), the case was considered non-informative. DNA sampled from separate epithelial and stromal cells demonstrating identical allelic loss pattern is compatible with either similar or independent clonal origin, whereas different patterns of allelic deletions are compatible with independent clonal origins of these tumors.[11–13,15,24,25]

Single-Stranded Conformation Polymorphism (SSCP) Analysis and p53 Immunostaining

Since mutations or functional inactivation of the p53 gene are the most common genetic abnormalities in cancer,[26] p53 analysis by immunohistochemistry as well as single-stranded conformation polymorphism (SSCP) were performed to determine whether mutations were present, and if so, whether the mutations were the same or different in the respective components. Genomic DNA was isolated from tissue using proteinase K digestion and phenol/chloroform extraction and subsequently amplified by PCR. The conditions for PCR were similar to the routine PCR except that 0.2 μl of α-[^{32}P]dATP (Perkin Elmer, Boston, MA) was added per reaction. Eight pair primers were chosen from exons 5–8 of the p53 gene. Twenty-five μl of PCR product was mixed with 3.5 μl of 95% formamide, 20 mmol/L EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol and heated at 95°C for 8 minutes. Two μl of this solution was loaded onto a 6% polyacrylamide gel with 10% glycerol. Gel electrophoresis was performed at 7 watts (W) for overnight. The gels were dried on filter paper and exposed to x-ray films at −80°C.[27,28]

P53 immunostaining was performed on formalin-fixed, paraffin-embedded sections using the avidin-biotin complex technique, as described previously.[29–31] Primary monoclonal antibodies were used for evaluation of p53 overexpression (DO-7, DAKO, Carpinteria, CA; dilution 1:100).

Statistical Analysis

Lack of association of allelic loss in the tumors between the epithelial and stromal sites would suggest independent origins of phyllodes tumor. Statistical analysis of the association was performed to confirm or disprove the hypothesis of independent origin.[32] In the analysis, response variable is allele loss in epithelial component and is a three-level categorical response, with 0 coded as no allele loss, 1 coded as loss of upper allele, and 2 coded as loss of lower allele. Homozygous controls (non-informative) response is not included in the analysis. The fixed predictor is allele loss in stromal component and is coded similarly as that of the response variable. Correlated multinomial logistic regression was performed; a random intercept for each case was used to account for correlation within a same case. Additionally, fixed or random effects of the twelve loci on the chromosomes within each case were included to account for the effect of the loci.

Results

The frequency of allelic loss is summarized in Figure 2. All patients with phyllodes tumor of the prostate showed allelic loss in both epithelial and stromal components (Table 1, Figure 3). All cases showed allelic loss in at least five loci when epithelial and stromal components are combined. The number of specific loci lost ranged from four to seven in the epithelial component and two to four in the stromal component.

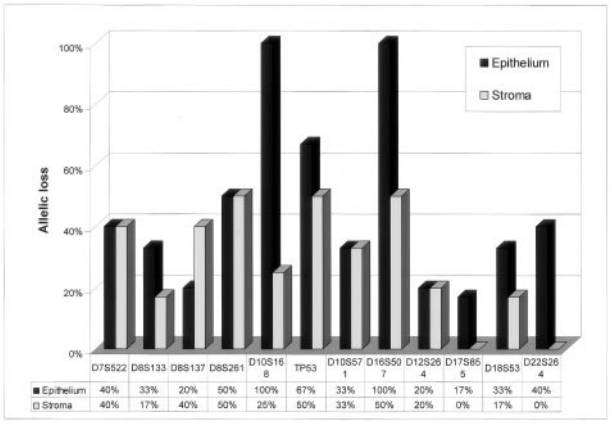

Figure 2. Comparison of frequency of allelic loss between epithelial and stromal components of phyllodes tumor.

Completely concordant allelic loss patterns between epithelium and stroma were not present in any of the tumors examined (Table 1). Case 2 showed allelic loss of four loci of both epithelial and stromal components, but only two of these loci (D8S137, D16S507) showed identical loss patterns. Similar results were seen in other cases, including case 3 with loss of seven epithelial and three stromal alleles, with complete concordance at three loci (TP53, D16S507, D12S264). Case 4 showed loss of four epithelial and two stromal alleles, with concordance at one locus (D16S507). Case 5 showed loss of five epithelial and three stromal alleles, with concordance at one locus (TP53). Case 6 showed loss of six epithelial and four stromal alleles, with complete concordance at two loci (D8S261, D18S53) and loss of opposite alleles at one locus (D10S168).

There were no completely identical allelic loss patterns when comparing epithelial and stromal components separately. High frequency of allelic loss of the epithelial component was seen at TP53, D10S168, and D16S507. In the epithelial component, 4 of 6 cases demonstrated identical allelic loss at TP53, 5 of 6 cases demonstrated identical allelic loss at D16S507 with the other having loss of the opposite allele, and 2 of 4 cases demonstrated identical allelic loss at D10S168 with the other two having loss of the opposite allele. The stromal component did not show as much similarity in allelic loss patterns and appeared more random, with only 3 of 6 cases showing identical allelic loss at TP53 and D16S507.

We further compared LOH patterns from different parts of the same tumor. These LOH findings were identical on multiple parts of specimens obtained from both left and right sides of a radical prostatectomy specimen (Case 6) (Figure 4). In three other cases where tumor was sampled from multiple sites, identical allelic loss patterns were also observed. Different parts of the same component (epithelial or stromal) yield the same LOH pattern (Figure 4).

P53 immunohistochemistry failed to reveal p53 protein overexpression in either epithelial or stromal components. Additionally, SSCP analysis did not demonstrate

Table 1. Composition of Allelic Loss in Matched Epithelial and Stromal Components of Phyllodes Tumors

Case	Microsatellite markers																			
	D7S522		D8S133		D8S137		D8S261		D10S168		D10S571		D12S264		D16S507		TP53		D17S855		D18S53		D22S264	
	E	S	E	S	E	S	E	S	E	S	E	S	E	S	E	S	E	S	E	S	E	S	E	S
1	▼	⬍	⬍	▼	⬍	▼	▼	⬍	▼	⬍	⬍	▼	⬍	⬍	▼	⬍	⬍	⬍	⬍	⬍	⬍	⬍	⬍	⬍
2	⬍	▲	⬍	⬍	▼	▼	⬍	▲	NI		⬍	⬍	NI		▼	▼	▲	⬍	⬍	⬍	▼	⬍	NI	
3	⬍	⬍	▼	⬍	NI		▼	⬍	▲	⬍	▼	⬍	▼	▼	▼	▼	▲	▲	⬍	⬍	⬍	⬍	⬍	⬍
4	▲	⬍	⬍	⬍	⬍	⬍	⬍	⬍	NI		⬍	▼	⬍	⬍	▼	▼	▲	⬍	⬍	⬍	⬍	⬍	▼	⬍
5	⬍	▲	⬍	⬍	⬍	⬍	⬍	▼	▼	⬍	▼	⬍	⬍	⬍	▼	⬍	▲	▲	⬍	⬍	⬍	⬍	▼	⬍
6	NI		▲	⬍	⬍	⬍	▲	▲	▲	▼	⬍	⬍	⬍	⬍	▲	⬍	⬍	▲	▲	⬍	▲	▲	⬍	⬍

E, Epithelial component of phyllodes tumor; S, stromal component of phyllodes tumor; ▲, loss of lower allele; ▼, loss of upper allele; ⬍, both alleles present; NI, noninformative.

any evidence of p53 mutations in either case where both alleles were present, or when one was lost.

From the statistical analysis using correlated multinomial logistic model, the upper allele loss in stromal component is not associated with the upper allele loss in epithelial component (P value = 0.48); the lower allele loss in stromal component is also not associated with the lower allele loss in epithelial component (P value = 0.28). This result indicates independent origin of epithelial and stromal components of the phyllodes tumors.

Discussion

In this study, we found a high frequency of allelic loss on chromosome 10q23, 16q23.2, and 17p13 in phyllodes tumor of the prostate. High frequency of allelic loss was found in the epithelial and stromal components, suggesting both components are clonal and neoplastic. The pattern of allelic loss is statistically different between the stroma and epithelium, supporting different clonal origins of the epithelial and stromal components of phyllodes tumor of the prostate.

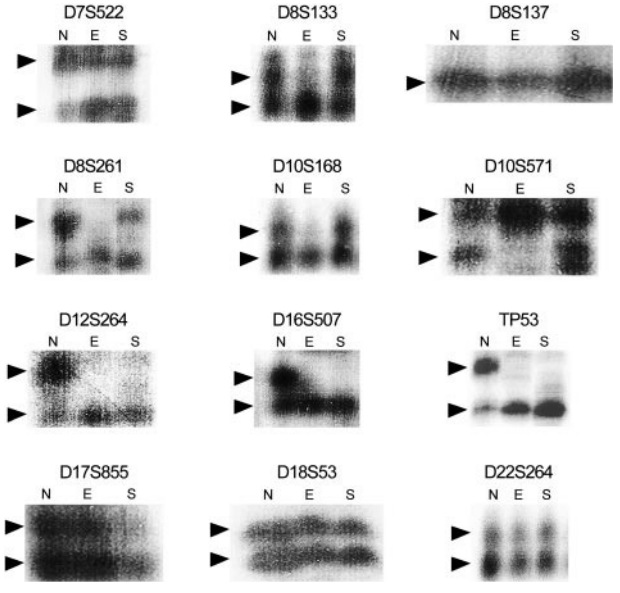

Figure 3. Representative results of loss of heterozygosity analysis (Case 3). DNA was prepared from normal tissue (N), epithelial (E), and stromal cells (S) of the phyllodes tumor, amplified by polymerase chain reaction using polymorphic markers D7S522, D8S133, D8S137, D8S261, D10S168, D10S571, D12S264, D16S507, TP53, D17S855 D18S53, D22S264, and separated by gel electrophoresis. **Arrows**, allelic bands.

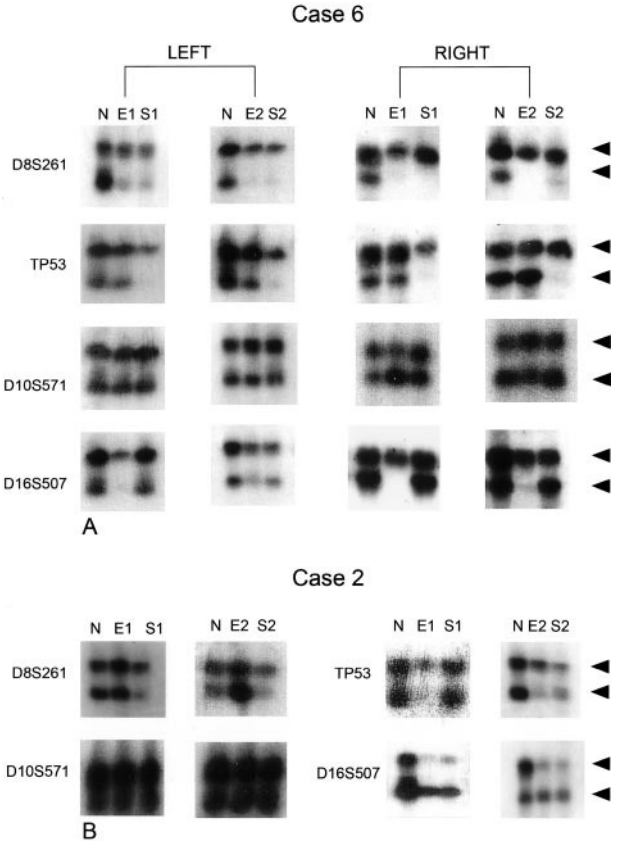

Figure 4. Different parts of the same component (epithelial or stromal) showed the same LOH pattern. DNA was prepared from normal tissue (N), epithelial (E), and stromal cells (S) of the phyllodes tumor and amplified by polymerase chain reaction. E1 and E2 represent epithelial components sampled from different tumor parts; S1 and S2 represent stromal components sampled from different tumor parts. The same LOH pattern was seen in tumor components of left and right sides of a radical prostatectomy specimen (Case 6) and multiple areas of different case (Case 2). **Arrows**, allelic bands.

Phyllodes tumor is a rare neoplasm of the prostate which may undergo sarcomatous transformation, is prone to early recurrences, infiltrative growth, and has potential for extraprostatic spread.[3–7] Benign clinical courses have been cited, but the follow-up time has been limited.[33] In regards to the stroma, Gaudin et al[34] proposed the clinicopathologic categories of prostatic stromal proliferation of uncertain malignant potential and prostatic stromal sarcoma to differentiate these entities from other mesenchymal lesions of the prostate, using immunohistochemical staining data in conjunction with morphological criteria.

Clonal evolution of transformed cell populations requires one or more genetic changes to obtain a growth advantage over adjacent cells. This results in the formation of a clinically detectable tumor. The specific types of genetic alterations associated with tumorigenesis are variable. They may range from DNA point mutations to major chromosomal structural aberrations or changes in chromosome numbers. Chromosomal analyses of phyllodes tumor of the breast have shown contradictory results. Noguchi et al[35] demonstrated the epithelial component to be polyclonal and the stromal component to be monoclonal. This led to the conclusion that phyllodes tumor is a neoplasm of stromal cells. However, Sawyer et al[36] demonstrated that allelic instability occurs in both the stroma and epithelia, indicating that both components are neoplastic.

The pathogenesis of phyllodes tumor of the prostate is unknown. This is the first study to assess loss of heterozygosity of this tumor. The high frequency of allelic loss of the epithelial component at 16q23.2 (D16S507) is striking. This locus encodes the gene HSD17B2, which is involved in steroid biosynthesis in the prostate. It has been suggested that the regulation of intraprostatic concentrations of active androgens is involved in the maintenance of organ homeostasis by modulating the balance between proliferation and apoptotic death of prostatic epithelial cells.[37] Recently, Harkonen et al[38] observed remarkable changes of 17HSD enzyme activities in prostate carcinoma, suggesting that this enzyme influences steroid hormone bioavailability locally and may contribute to the progression of prostate cancer.

The frequent epithelial allelic loss of p53 (TP53) and PTEN/MMAC1 (D10S168) suggests phyllodes tumor development may occur through inactivation of tumor suppressor genes, as has been shown in prostate carcinoma.[29–31,39,40] Our data showed detection of allelic loss as a poor indicator of mutation of the remaining copy of the p53 gene. This is not an uncommon finding as Brooks et al[39] had one of 10 tumors in their series of prostatic carcinoma with a mutation of its remaining allele documented using SSCP, and none of the tumors with allelic loss were immunohistochemically positive. This lack of concordance between 17p allelic loss and p53 mutation has been occasionally observed in bladder and head and neck tumors and more commonly in breast cancers and astrocytomas.[41–44]

In summary, our study demonstrated that phyllodes tumors of the prostate are clonal proliferations. Epithelial and stromal components may have different clonal origins and appear to be true neoplastic conditions. Our data support that the epithelial and stromal components may arise independently and provide support for a pathogenetic model of phyllodes tumor of the prostate where both epithelial and stromal components are neoplastic. However, given that there are occasional similarities in LOH patterns, although statistically insignificant, one must also consider common clonality with loss of additional alleles separately after divergence of the two components.

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