

COMBINED POLYMERASE CHAIN REACTION (PCR) AND FLUORESCENCE IN SITU HYBRIDIZATION (FISH) ANALYSIS OF Y CHROMOSOME LOSS RELATED TO AGE AND DISEASE STATUS IN PROSTATE CANCER

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SUMMARY

Aneusomy of the Y chromosome was examined in paired malignant and benign prostate tissue from 31 radical prostatectomy specimens to analyze the possible relationship between Y chromosome loss and disease status, and to define its relationship to the aging process. Tissues from men aged 45-55 years or 56-75 years at the time of prostate cancer diagnosis were examined for Y chromosome dosage using Polymerase Chain Reaction (PCR) and Fluorescence In Situ Hybridization (FISH) techniques.

PCR analysis of malignant tissue demonstrated Yp sequence losses in 0 % (0/20) of men aged 45-55 years and 18% (2/11) of men aged 56-75 years; no losses were observed in benign epithelium from either age group. FISH analysis demonstrated Y chromosome loss in 5% (1/20) of malignant tumors, 14% (1/7) of prostatic intraepithelial neoplasia (PIN) lesions, and 0% (0/20) of benign glands in men aged 45-55 years, and in 18% (2/11) of malignant glands, 50% (1/2) of PIN lesions and 9% (1/11) of benign glands in men aged 56-75 years. Interestingly, focal loss of the Y chromosome involving multiple or single malignant glands was observed by FISH analysis in 25% (5/20) of men aged 45-55 years and 1/11 (9%) of men aged 56-75 years. These results indicate that Y chromosome loss is associated with both the aging process and malignancy in the prostate.

Key Words : Prostate Neoplasms, Y Chromosome, In Situ Hybridization, Polymerase Chain Reaction

INTRODUCTION

Carcinoma of the prostate (CaP) is the second leading cause of cancer death among American males, and its incidence is steadily increasing (1).

The genetic alterations responsible for prostate tumorigenesis are not yet clearly defined, but are assumed to comprise gain or loss of specific chromosomal regions or whole chromosomes (2,3). However, the relationships between genetic aberrations, diagnosis and/or ultimate outcome are unknown. The karyotypic characteristics of CaP are complex, and point to few, if any specific chromosomal changes. In addition, histopathologic heterogeneity of the prostate cancer may be reflected at the genetic level (3,4). Although no single specific chromosomal change has been established with certainty in prostate cancer, loss of the Y chromosome and the 7q, 8p, 10q and 16q chromosomal regions have been reported (2,5,6).

Y chromosome loss is known to occur as a result of normal aging process (7), and may be due to existing mosaicism within the normal tissue, with or without the aging process (7-9). However, Y chromosome loss has also been identified in 5-20% of malignancies, including CaP (2,10-17). Since the majority of these studies utilized tissue culture techniques, the intriguing question of whether the observed chromosomal aberrations were generated in vitro, remains to be answered (7).

This study was undertaken to define the possible changes of Y chromosome status in prostate cancer by direct examination of pathologic specimens with PCR and FISH techniques, and to define the relationship between the patient age and disease state in the prostate.

MATERIAL AND METHODS

Surgical specimens from a total of 31 patients following radical prostatectomy were examined. Patient characteristics of the study group in terms of age, stage and grade are summarized in table I.

Prostate tissues were processed as previously reported (2,3). Briefly, the capsular surface of specimens was stained with India ink and serially sectioned at 3-5 mm intervals. The tissue samples were subsequently routinely formalin-fixed and paraffin embedded. Areas of benign prostatic hyperplasia (BPH), prostatic intraepithelial neoplasia (PIN), and adenocarcinoma of the prostate (CaP) were encircled with ink on slides stained with Hematoxylin and Eosin to ensure characterization of the lesion, and used as templates to microdissect tissue for PCR or to perform FISH analysis of the same lesions from adjacent, non-stained slides.

PCR

After the entire tissue section was deparaffinized in xylene, circumscribed areas were excised and digested with proteinase K. Commercial reagents and protocols (Perkin Elmer Cetus) were used to perform a coamplification of the ZFY, and human beta globin (HBB) locus sequences in the presence of 10 MCi of alpha³²PdCTP (> 3000 Ci/mmol) (2,3,18). Oligonucleotide primers were used to amplify sequences that map to Yp11.3 (ZFY locus, Genebank accession JO3134), and 11p15.5 (HBB locus, used as quantitation control, combined Genebank accession numbers), as previously described (2, 3). Reaction products were electrophoresed on 8% acrylamide/7 M urea sequencing gels and autoradiographed. Quantitation was accomplished by scanning laser densitometry and by comparison of the signal intensities of the test

locus to that of the control (HBB). Dosage of 11p sequences were also controlled by separate amplification of highly polymorphic sequences at the D11S860 locus at 11p15 (19).

FISH

Slides were deparaffinized then hybridized with biotin-labelled specific probes from a chromosome in situ kit (Oncor, Gaithersburg, MD). Two types of probes were included in a cocktail: Classical satellite probes specific to short AATGG related repeats localized near the centromere in heterochromatic areas of the long arm of the Y chromosome, and alpha satellite probes specific to the highly repeated alphoid DNA which are tandem monomeric repeats of 171 bp in length, located at the centromere of Y chromosome. Detection of the probes utilized fluorescein labelled-avidin, and slides were counterstained with propidium iodide, then analyzed on a Zeiss epifluorescence microscope using a dual-pass filter, at 100 to 1000 magnifications (17).

An average of 400 cells per lesion, from at least 4 different quadrants, were counted to obtain a thorough sampling. Each of the glandular structures were counted and noted separately. Complete (overall) loss of the Y chromosome was described as the loss in 50% or more of all cells counted. Nodular (focal) loss was scored when 50% of the cells within a single malignant gland demonstrated loss of the Y chromosome.

Table I- Distribution of patients according to type of malignant and non-malignant prostate lesions

AGE	NUMBER OF PATIENTS							
	STAGE				GRADE			
	T1	T2	T3N0	T3N+	4 - 6	7 - 9	PIN	BPH*
45 - 55	1	11	7	1	4	16	7	20
56 - 75	ND	1	6	4	2	9	2	11
TOTAL	1	12	13	5	6	25	9	31

* Includes hyperplastic and occasional non-hyperplastic benign epithelium.

RESULTS

Analysis of paired malignant and benign tissue samples from radical prostatectomy specimens by PCR demonstrated Yp sequence losses in 7% (2/31) of malignant tumors within the entire study group, without any loss in benign epithelium or PIN. All losses were observed in the older age group and were confined to locally advanced (T3NO) CaP cases.

FISH studies within the entire study group demonstrated aneusomy of Y chromosome in 10% (3/31) of malignant tumors and in 3% (1/31) and 22% (2/9) of BPH and PIN lesions, respectively. FISH analysis demonstrated chromosome loss in 5% (1/20) of malignant tumors, 14% (1/7) of prostatic intraepithelial neoplasia (PIN) lesions, and 0% (0/20) of benign glands in men aged 45-55 years, and in 18% (2/11) of malignant glands, 50% (1/2) of PIN lesions and 9% (1/11) of benign glands in men aged 56-75 years. Y chromosome loss in younger men was confined to a stage T2 tumor and a PIN lesion. In older men, Y chromosome loss was detected in T3NO (1/6, 17%), T3N+ (1/4, 25%) tumors, PIN (1/2, 50%) lesions and BPH (1/11, 9%). The majority of all tumors examined were poorly differentiated with a combined Gleason score of 7 to 9 (25/31, 81%). Therefore, no conclusion could be made regarding the correlation between the tumor grade and PCR or FISH analysis.

Loss of Y chromosome in a "nodular" fashion was observed in malignant tumors, but not in BPH (Fig. 1, Table II). This particular type of focal loss was seen in 19 % (6/31) of all patients, and its frequency was much higher in younger patient group compared to older ones (25% and 9% respectively). Interestingly, nodular Y chromosome loss in younger men was 3 times more frequent in tumors that were confined to prostate gland (31 %) than extraprostatic disease (11%). Nodular Y chromosome loss was also observed in PIN lesions from both age groups (Table II).

When the results of PCR and FISH methods were combined, the detection rate of absolute Y chromosome loss increased to 16% (5/31), compared to 7% (2/31) by PCR and 10% (3/31) by FISH techniques alone. If nodular losses were also considered, combination of both methods was able to demonstrate Y chromosome loss in 35% (11/31) of the cases.

DISCUSSION

Cytogenetic analysis of primary CaP in recent studies (6,14,15,17) identified the loss of Y chromosome as

the most common numerical aberration (clonal loss in 5-20% of the tumors), and it has been suggested that the presence of clonal karyotypic changes correlated in general with a poorly differentiated state of cancer, and may be an independent prognostic factor (20,21). However, the results of another study utilizing reverse transcriptase PCR and Southern blotting, indicated that at least the portion of Y chromosome which encodes zinc-finger DNA binding protein (ZFY) was not lost from the majority of CaP cells (22).

The application of PCR and FISH techniques revealed overall Y chromosome loss in 7% and 10%, respectively, of tumors examined. These numbers are comparable to those reported using conventional metaphase analysis of short term primary prostate tissue cultures (6,14,15,17). Also, our results indicate that loss of Y chromosome in these studies was not an "artifact" of cell culture, since we observed similar results using PCR and FISH analysis of uncultured tissue. Therefore, Y chromosome loss characterizes some prostate tumors in vivo.

Both PCR and FISH provide information about Y chromosome dosage in a specified lesion. Their concurrent utilization proved to be a more sensitive measure of Y chromosome number than either method alone, because concurrent application of PCR and FISH to prostatic tissue has yielded evidence of higher frequencies of overall or nodular Y chromosome loss in CaP (35%) than did either method alone (7% by PCR and 29% by FISH).

Specimens from older patients displayed overall loss of the Y chromosome almost 4 times more frequently than younger patients (18% vs 5%). Thus, overall Y chromosome loss appears to be associated with aging process and malignancy in prostate. In contrast, patients below the age of 55 demonstrated nodular Y chromosome loss 3 times more frequently than older patients. Therefore, with increasing patient age and tumor stage, we observed a shift from a partially expressed chromosomal abnormality (nodular loss) to an extensive one (dispersed loss, involving at least 50% of the cells in a given lesion) in prostate tumors.

From our data, we may hypothesize that Y chromosome loss may initiate in a sporadic fashion, relatively early in the course of disease; this may appear as the "nodular" loss we have observed. The involvement of more and more glands into nodular loss arrays as tumor progress, may then result in a more dispersed, extensive pattern of Y chromosome loss, such as we observed in older patients with more advanced disease. Further studies should reveal whether this "pattern" of loss is unique to the Y chromosome, or characterizes somatic chromosomes, as well, in human malignancies.

Table II- Details of nodular (focal) loss of the Y chromosome by FISH analysis

AGE	CaP Confined into Capsule (T1 - T2)	Extracapsular CaP (T3/N+)	PIN	BPH
45 - 55	4/12 (33%)	1/8 (13%)	1/7 (14%)	0/20
56 - 75	0/1	1/10 (10%)	1/2 (50%)	0/11
TOTAL :	4/13 (31 %)	2/18 (11%)	2/9 (22%)	0/31

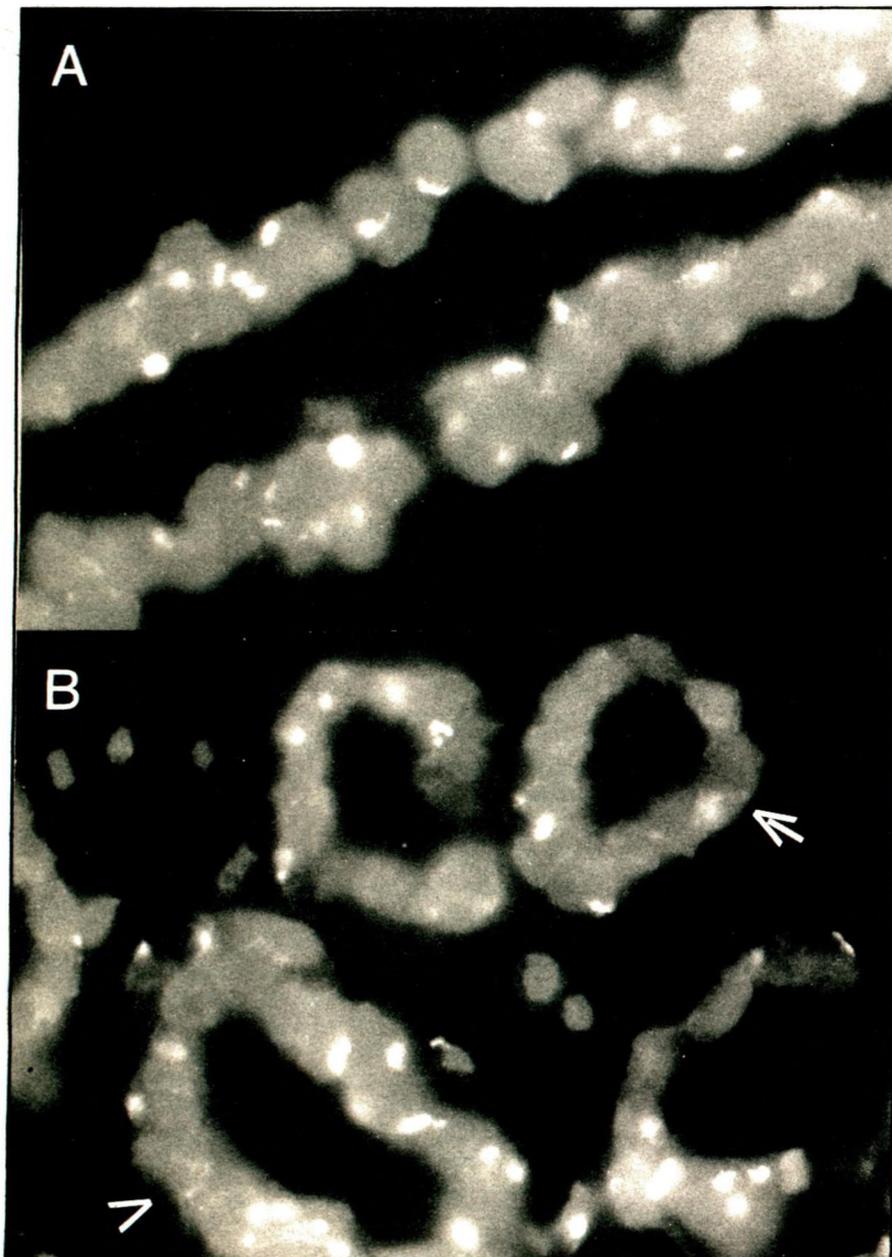


Fig 1. FISH analysis of Y chromosome in benign and malignant prostate tissue. (A) Hybridization with a Y chromosome specific probe cocktail in benign glands demonstrates the frequent occurrence of nuclei with a single, bright, peripheral signal. In CaP tissue (B), the majority of tumor cells within glands on the left quadrant (arrow head) possess the Y chromosome, whereas the majority of the cells in the upper right CaP gland (arrow), have deleted the Y chromosome. This result demonstrates nodular loss of the Y chromosome within a single tumor focus. (Magnification X 1000).

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