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# DNA Sequence Amplification in Human Prostate Cancer Identified by Chromosome Microdissection: Potential Prognostic Implications[1]

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## ABSTRACT

**The primary aim of this report was to examine the significance of increased DNA sequence copy number (gene amplification) in human prostate cancers. Three methodologies (chromosome microdissection, comparative genomic hybridization, and fluorescence *in situ* hybridization) were combined to (*a*) identify a common region of gene amplification in human prostate cells and (*b*) evaluate in patient samples the prevalence of this genetic change in both primary and recurrent prostate samples. The results of chromosome microdissection revealed a common amplified band region (8q24.1-24.2) in two prostate cases with cytological evidence of gene amplification (double minutes). Fluorescence *in situ* hybridization using the 8q microdissection probe was performed on fresh tumor touch preparations from 44 randomly selected prostatectomy specimens. Amplification of DNA sequences from 8q24 was observed in 4 (9%) of 44 cases. Four of the 44 patients in this series presented with a positive lymph node at initial diagnosis and 3 of these 4 patients showed 8q amplification. Because of this finding, comparative genomic hybridization and fluorescence *in situ* hybridization were performed on tumor cells from nine prostate cancer patients with recurrent disease. In eight of nine cases a gain of DNA sequences encompassing 8q24 was observed. Taken together with other evidence implicating 8q gain in prostate cancer progression, these results suggest that the analysis of this genetic change may have diagnostic utility as a marker of prostate cancer progression.**

## INTRODUCTION

Although prostate cancer is the most commonly diagnosed cancer in men in the United States (~200,000 newly diagnosed cases/year; Ref. 1), the molecular changes underlying its genesis and progression remain poorly understood. With the extreme variability in the natural history of this disease, coupled to frequent incidental diagnosis of subclinical disease [following PSA testing or transurethral resection for urinary obstruction (2)], the identification of genetic markers which could identify prostate cancers likely to progress to lethal metastatic disease is an important goal.

Recent studies have identified several recurring genetic changes in prostate cancer including: (*a*) allelic loss, particularly loss of chromosome 8p and 16q (3–5); (*b*) generalized DNA hypermethylation (6); (*c*) point mutations or deletions of the retinoblastoma and *p53* genes (7–9); and (*d*) alterations in the level of specific cell to cell adhesion molecules (*i.e.*, E-cadherin/α-catenin; Refs. 5, 10, and 11) and aneuploidy and aneusomy of chromosomes detected by FISH[4], particularly chromosomes 7 and 8 (4, 12–15). It seems certain that a combination of these changes is critical to the acquisition of metastatic potential, and Issacs *et al.* (6) have recently proposed a model placing these genetic changes in the context of prostate cancer disease progression.

At the current time, a method to accurately predict the metastatic spread of an individual tumor is not available. Historically, clinical and pathological stage and histological grading systems (*e.g.*, Gleason's) have been used to indicate the prognosis for groups of patients based on the degree of tumor differentiation or the type of glandular pattern (16, 17). The use of a computer system image analysis of histological sections of primary lesions has also been suggested as an aide in the management of individual patients (17). Additionally, DNA content/ploidy by flow cytometry (18) and, very recently, FISH using centromere-specific probes have also been demonstrated to have utility as a marker of prostate cancer aggressiveness (4, 12–15, 18). Finally, the use of CGH has very recently been performed on prostate cancer specimens (19, 20), enabling investigators to survey the entire genome for gains and losses of DNA sequences, thus pinpointing chromosomal regions likely to contain genes implicated in the development or progression of prostate cancer.

Finally, for many cancers the presence of gene amplification has been documented to represent an important prognostic indicator of disease progression. Cytological evidence of gene amplification (recognized as extrachromosomal dmin) has been previously reported in prostate tumor specimens (21–23), al-

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[4] The abbreviations used are: FISH, fluorescence *in situ* hybridization; CGH, comparative genomic hybridization; dmin, double minutes; PCR, polymerase chain reaction; PSA, prostate-specific antigen.

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though no evidence of gene amplification of known oncogenes has yet been reported. The identification of a commonly amplified chromosome region in prostate cancer would be of considerable importance. The presence of dmin in metaphase spreads from short-term prostate cultures provides a target for the technique of chromosome microdissection. This procedure uses a glass microneedle to dissect (under the microscope) dmin and amplify this minute amount of DNA via PCR (24). The product is then hybridized to normal-banded lymphocyte metaphase chromosomes to determine the chromosomal location of the amplified genes (25, 26). Using this approach, we have identified in the current study a common chromosome band region amplified in prostate cancer (8q24.1-24.2).

This finding led to the design of our current study with three specific goals: (*a*) to generate a chromosome microdissection probe suitable for FISH encompassing the 8q24 amplified region in dmin-bearing prostate tumor metaphase cells; (*b*) to utilize this probe in FISH analysis of interphase nuclei to determine the frequency of DNA copy number increases in an unselected series of primary prostate lesions; and (*c*) to utilize CGH to assess the DNA copy number changes for 8q in prostate cancer patients with recurrent disease. The underlying presumption of this work is that the overexpression by gene amplification of a gene(s) on chromosome 8 may prove to be a biologically and prognostically important factor in assessing prostate cancer progression.

## MATERIALS AND METHODS

**Surgical Specimens.** Tissue from primary disease specimens for FISH or microdissection was obtained from patients undergoing radical prostatectomy at the Mayo Clinic, Texas Health Science Center of San Antonio, or Johns Hopkins University. All samples were obtained prior to administration of any hormonal therapy, and all as part of their regular clinical care. All recurrent specimens came from Tampere University Hospital following transurethral resections of patients who had responded favorably to endocrine therapy (orchiectomy, six cases; luteinizing hormone-releasing hormone agonist, two cases; estrogen, one case), but later showed symptoms of local recurrence.

**Preparation of Metaphase Chromosomes for Microdissection.** In order to obtain metaphase chromosomes for microdissection, cells were analyzed from a short-term culture of a primary tumor specimen (PRO-39) and from an established prostate cancer cell line (MPC-3).

Primary tumor tissue was obtained at the time of radical prostatectomy from case PRO-39 and a portion of the tumor was used for determination of histology and standard pathology. The specimen was obtained from a 63-year-old male. The pathological findings concluded 45% of the prostate gland was involved by moderately to poorly differentiated adenocarcinoma, Gleason's grade 8/10 (5+3/10) (Table 1) as: (*a*) poorly differentiated adenocarcinoma (primary pattern 5 in Gleason's grading system) and (*b*) moderately differentiated adenocarcinoma (secondary pattern 3 in Gleason's grading system). Only 1 of 13 lymph nodes examined demonstrated metastatic adenocarcinoma. Prostate tumor chips were transported in McCoy's medium with 10% fetal bovine serum, 2% HEPES buffer, 500 units/ml penicillin/streptomycin, and 2 mM sodium pyruvate. Tissue for cytogenetic analysis was minced into 1–2-mm$^3$ fragments, then forced through a 100 mesh sieve. A single-cell suspension was made by passing cells through a 22½-gauge needle. Cells were centrifuged and washed, then resuspended in McCoy's 5A medium as described above. Cells were treated with colcimid (0.12 μg/ml) for 60 min. Cells were removed from the flask using 0.25% trypsin/ml mM EDTA, resuspended in 0.075 M KCl at room temperature for 20 min, centrifuged and subsequently fixed in 3:1 (methanol:glacial acetic acid). After overnight fixation at −20°C, the cells were refixed in fresh fixative and metaphase spreads were stained with Wright's stain as described previously (27).

The MPC-3 prostate tumor cell line, previously reported to contain dmin and possess c-*myc* amplification (28, 29), was grown in RPMI 1640 medium with 10% fetal bovine serum and glutamine. MCP-3 cells were exposed to colcimed, harvested, fixed, and prepared on slides as described above.

**Microdissection and Amplification of dmin DNA.** The microdissection and PCR amplification of dissected dmin DNA were performed essentially as described previously (30). Briefly, microdissection was performed using glass microneedles controlled by a micromanipulator attached to an inverted microscope. The target region of dissection in both the primary specimen and the prostate cell line were 3–5 of dmin. The dissected dmin fragments (which adhere to the microneedle) were transferred to a 5-μl collection drop and amplified using previously published methods for degenerate oligonucleotide-primed PCR. The amplified microdissected DNA was labeled with biotin-11-dUTP in a secondary PCR reaction and the products were purified and used for FISH. Hybridization of the FISH probes followed previously described procedures (24) and provided unequivocal evidence of hybridization to the dmin DNA.

**FISH Analysis of Tumor Tissue Specimens.** Dual-color hybridizations with a directly labeled probe for the centromere of chromosome 8 (Spectrum Green CEP-8; VYSIS, Framingham, MA) and the directly labeled Spectrum Orange 8q24 probe were performed on prostate tumor touch preparations as previously described (18). Posthybridization conditions and methods of microscopic analysis have also been previously reported (18).

For each batch of hybridizations a negative control (benign prostate tissue at Mayo, peripheral blood lymphocytes at Hopkins) was hybridized and scanned to demonstrate successful and appropriate normal hybridization signals. In the normal control and in the apparently 8q24 nonamplified tumor cases, one small, distinct orange-red signal (from the 8q24 probe) was observed to be associated with each of the larger green signals (the CEP 8 probe) in ≥92% of cells. For each batch of hybridizations a positive control (the MPC-3 cancer cell line) was also hybridized and scanned to demonstrate successful and appropriate amplification signals. Amplification of 8q24 was defined by multiple (often diffuse) orange-red signals associated with one or more of the green signals or as large orange-red signal domains within a nucleus. The observation of four 8q24 signals and two CEP8 signals (*e.g.*, four copies of the 8q arm or 8q24 and two copies of the chromosome 8 centromere) was not defined as amplification. Whenever possible, both 8 chromo-

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*Table 1* Patient information

| Patient | Institution | Clinical state | Pre-Op[a] PSA value | Post-OP pathological staging | Gleason's primary | Score secondary | Differentiation (poor, medium, well) (Mayo 3, 2, 1) | Ploidy FCM | 8q Probe results (%)[b] | Tumor size (cm) |
|---|---|---|---|---|---|---|---|---|---|---|
| 24 | Mayo | C | 13.40 | 3a-0-0 | 2 | 3 | 2 | Diploid | Neg | 4.2.2 |
| 32 | Mayo | B | 1.00 | 2a-0-0 | 2 | 3 | 2 | Diploid | Neg | 2 × 1 × 1 |
| 20 | Mayo | C | 6.20 | 3a-0-0 | 3 | 4 | 3 | Diploid | Neg | 2 × 1 × 1 |
| 11 | Mayo | C | 4.00 | 3a-0-0 | 3 | 3 | 2 | Diploid | Neg | 2.5 × 1.7 × 3.5 |
| 13 | Mayo | B | 5.60 | 2c-0-0 | 3 | 4 | 3 | Diploid | Neg | 2.1 × 1.5 × 0.5 |
| 5 | Mayo | B | 7.80 | 2c-0-0 | 3 | 2 | 2 | Diploid | Neg | 3 × 3 × 1 |
| 16 | Mayo | C | 4.10 | 3a-0-0 | 3 | 3 | 2 | Diploid | Neg | 2.4 × 1.8 × 1 |
| 1 | Mayo | B | 19.70 | 2c-0-0 | 3 | 3 | 3 | Diploid | Neg | 2 × 2 × 1 |
| 17 | Mayo | C | 4.20 | 3c-0-0 | 3 | 2 | 2 | Diploid | Neg | 2.5 × 2.5 × 1.3 |
| 51 | Mayo | B | 11.70 | 2c-0-0 | 2 | 3 | 2 | Diploid | Neg | 4.2 × 4 × 3.9 |
| 10 | Mayo | C | None | 3a-0-0 | 3 | 3 | 3 | Diploid | Neg | 1.5 × 1.5 × 1 |
| 28 | Mayo | C | 22.60 | 3b-0-0 | 3 | 3 | 3 | Diploid | Neg | 3.5 × 3 × 1.3 |
| 6 | Mayo | B | 6.20 | 2c-0-0 | 2 | 2 | 2 | Diploid | Neg | 0.8 |
| 2 | Mayo | C | 8.50 | 3a-0-0 | 3 | 3 | 3 | Tetraploid | Neg | 2.5 × 2 × 1 |
| 34 | Mayo | C | 6.10 | 3-0-0 | 3 | 4 | 3 | Diploid | Neg | 2.3 × 1.8 × 0.8 |
| 23 | Mayo | C | 3.90 | 3b-0-0 | 3 | 3 | 3 | Diploid | Neg | 4 × 2.8 × 1.1 |
| 39 | Mayo | B | 4.60 | 2c-0-0 | 3 | 3 | 2 | Tetraploid | Neg | 2 × 2 × 1 |
| 31 | Mayo | C | 5.70 | 3a-0-0 | 2 | 3 | 2 | Tetraploid | Neg | 2.2 × 1.7 × 0.9 |
| 9 | Mayo | B | 5.70 | 2b-0-0 | 3 | 5 | 3 | Tetraploid | Neg | 2 × 2 × 1 |
| 8 | Mayo | C | 3.10 | 3a-0-0 | 3 | 3 | 3 | Diploid | Neg | 1.5 × 1 × 0.8 |
| 18 | Mayo | C | 35.60 | 2b-0-0 | 3 | 2 | 3 | Tetraploid | Neg | 2.5 × 2 × 1.5 |
| 19 | Mayo | B | 4.90 | 2b-0-0 | 3 | 4 | 3 | Tetraploid | Neg | 2 × 2 × 1 |
| 21 | Mayo | C | 19.20 | 3a-0-0 | 3 | 4 | 3 | Tetraploid | Neg | 2 × 1.5 × 1.5 |
| 25 | Mayo | C | 26.60 | 3c-0-0 | 3 | 4 | 3 | Tetraploid | Neg | 4 × 3.5 × 2.5 |
| 33 | Mayo | C | 4.50 | 3c-1-0 | 3 | 3 | 3 | Tetraploid | 10.50% | 4.5 × 3 × 2 |
| 35 | Mayo | C | 16.70 | 3c-0-0 | 2 | 4 | 2 | Diploid | Neg | 3.5 × 2 × 1.2 |
| 37 | Mayo | C | 5.70 | 3c-0-0 | 3 | 4 | 3 | Aneuploid | 18.50% | 4 × 3.5 × 3 |
| 43 | Mayo | C | 9.30 | 3c-0-0 | 3 | 4 | 3 | Tetraploid | Neg | 2.5 × 2 × 1.3 |
| 46 | Mayo | C | 9.30 | 3-0-0 | 3 | 3 | 3 | Tetraploid | Neg | 5 × 3 × 1.5 |
| 48 | Mayo | C | 6.70 | 3a-0-0 | 3 | 3 | 3 | Tetraploid | Neg | 2.5 × 2 × 2 |
| 49 | Mayo | C | 28.30 | 4a-0-0 | 3 | 3 | 3 | Aneuploid | CEP8/8q24<1 | 4 × 4 × 3.3 |
| 53 | Mayo | C | 8.80 | 3c-0-0 | 3 | 3 | 3 | Diploid | Neg | 2.5 × 2.2 × 1.5 |
| 94-084 | Hopkins | B | 22.50 | 3c-1-0 | 4 | 4 | 3 | Aneuploid | 30% | 1.5 × 0.5 × 0.5 |
| 94-007 | Hopkins | B | 7.60 | 3a-0-0 | 3 | 3 | 2 | ND | Neg | 1 × 1 × 1 |
| 94-002 | Hopkins | B | 3.70 | 2b-0-0 | 3 | 3 | 2 | ND | Neg | 1 × 1 × 0.75 |
| 94-008 | Hopkins | B | 8.90 | 2b-0-0 | 3 | 4 | 2 | Aneuploid | Neg | 1 × 1 × 1 |
| 94-027 | Hopkins | B | 10.10 | 2a-0-0 | 3 | 3 | 2 | ND | Neg | 2 × 1 × 1 |
| 94-089 | Hopkins | B | 1.00 | 2a-0-0 | 3 | 4 | 2 | Diploid | Neg | 1 × 1 × 1 |
| 94-044 | Hopkins | B | 6.40 | 2a-0-0 | 4 | 3 | 3 | Diploid | Neg | 1 × 1.5 × 0.7 |
| 94-024 | Hopkins | B | 4.30 | 2c-0-0 | 4 | 4 | 3 | Diploid | Neg | 2 × 3 × 1.5 |
| 94-020 | Hopkins | B | 21.80 | 2a-0-0 | 3 | 3 | 2 | Aneuploid | Neg | 2 × 2 × 1 |
| 94-049 | Hopkins | B | 12.80 | 2b-0-0 | 3 | 3 | ND | Diploid | Neg | 1 × 1 × 1 |
| PRO-39 | UTSA | B | 31.00 | 3b-1-0 | 5 | 3 | ND | ND | Amplified | ND |

[a] Pre-Op, preoperative; Post-Op, postoperative; Neg, negative, ND, not determined.
[b] Percentage of nuclei demonstrating amplification.

some hybridization and the 8q micro-FISH probe were simultaneously assayed. For each of the prostate tumors studied, the entire hybridization site (22 × 22 mm) was analyzed. All nuclei encountered while scanning were carefully evaluated for the presence of amplification. If amplifications were observed following this complete scan, 200–250 random nuclei were scored and the percentage of nuclei demonstrating amplification was recorded (Table 1).

**Comparative Genomic Hybridization.** CGH was performed on three prostate cell lines (du145, LNCap, and PC-3) and nine recurrent tumors using directly fluorochrome-conjugated DNAs as described previously (31, 32). Briefly, normal lymphocyte metaphase preparations were denaturated at 72–74°C for 3 min in 70% formamide-2X SSC (pH 7), and dehydrated in a series of 70, 85, and 100% ethanol followed by proteinase K (0.1 mg/ml in 20 mM Tris-HCl, 2 mM $CaCl_2$, pH 7.5) treatment at room temperature and a second round of dehydration as described above. DNA isolated from tumors was labeled with FITC-dUTP (DuPont, Boston, MA) and normal male DNA with Texas Red-dUTP (DuPont) using nick translation. Hybridization mixture containing 400 ng labeled tumor and normal DNA and 10 mg unlabeled Cot-1 DNA (GIBCO-BRL, Gaithersburg, MD) in 10 ml of 50% formamide-10% dextran sulfate-2X SSC was denaturated and applied on normal lymphocyte metaphase preparations. The hybridization was done under a coverslip in a humid chamber at 37°C for 48 h. After hybridization, the slides were washed three times in 50% formamide-2X SSC (pH 7), twice in 2X SSC, and once in 0.1X SSC at 45°C for 10 min each followed by 2X SSC, 0.1 M $NaH_2PO_4$-0.1 M $Na_2HPO_4$-0.1% NP40 (pH 8), and distilled

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water at room temperature for 10 min each. The slides were then counterstained with 4,6-diamidino-2-phenylindole (0.1 mg/ml) in an antifade solution. Hybridizations of FITC-labeled normal female DNA with Texas Red-labeled normal male DNA were used as controls.

**Digital Image Analysis.** Detection of relative DNA sequence copy number changes in chromosome 8 was accomplished by analyzing hybridization intensities of tumor and normal DNAs along chromosome 8 in metaphase spreads using a digital image analysis system as described previously (32). Three single-color images (matching 4,6-diamidio-2-phenylindole, FITC, and Texas Red fluorescence) were collected from four to five metaphases from each hybridization using a Nikon SA epifluorescence microscope (Nikon Corporation, Tokyo, Japan) and a Xillix CCD camera (Xillix Technologies Corporation, Vancouver, British Columbia, Canada) interfaced to a Sun LX workstation (Sun Microsystems Computer Corporation, Mountain View, CA). After background subtraction, the green and red fluorescence intensities from pter to qter and the ratio of the two were calculated. The absolute fluorescence intensities were normalized for each metaphase spread so that the average green:red ratio of all chromosome objects in the metaphase was 1.0. The final result was plotted as a mean green:red ratio profile and its $\pm 1$ SD from pter to qter along chromosome 8 obtained from 4–8 chromosome homologues.

## RESULTS

**Identification of Gene Amplification in Prostate Cells by Chromosome Microdissection.** We have recently developed a strategy to identify and characterize gene amplification by targeting dmin or hsrs for microdissection (25, 26). In this study we report the results of microdissecting dmin from two prostate cancers, identifying a common origin of amplified DNA in both cases, and using the microdissection probe to test interphase tumor cells from patient samples.

Dmin from the primary tumor case PRO-39 and from the MPC-3 prostate cancer cell line were microdissected (Fig. 1, *A-C*) and directly amplified *in vitro* by PCR. The PCR-amplified DNA sequences were then secondarily labeled with a fluorochrome and used for FISH against metaphase tumor cells from which the dissections were derived (Fig. 1, *D* and *E*). The results of FISH to both dmin-bearing tumor cell metaphases clearly documented hybridization to the dmin, confirming the presence of DNA sequence amplification (Fig. 1*E*). Subsequently, the dmin microdissection probe was used to hybridize against metaphase spreads from normal peripheral blood lymphocytes in order to identify the chromosomal loci of the amplified DNA sequences encoded within the dmin (Fig. 2, *A* and *B*). The origin of the amplified DNA within PRO-39 dmin was shown to encompass band region 8q24 (Fig. 2). The dissected dmin DNA from MPC-3 was shown to hybridize to two different band regions, 8q24 and 10cen (Fig. 2, *C* and *D*).

The PRO-39 microdissection probe was then hybridized to MPC-3 metaphases and hybridization was visable on 100% of the recognizable dmin. A biotin-labeled c-*myc* cosmid was then hybridized to mitoses from PRO-39, PC-3, and MPC-3. In all three cases, c-*myc* sequences were shown to hybridize to all dmin (Fig. 1*E*, *inset*).

**FISH Analysis of Prostate Tumor Specimens.** Based on the identification of a commonly amplified band region (8q24), dual-color FISH (using a directly labeled probe for the centromere of 8, labeled green, and the dissected band region 8q24, labeled orange-red) was performed on touch preparations from the tumors of 44 patients with prostate cancer. Table 1 summarizes the clinical information and amplification status of 8q24 amplification of all 44 patients. Within this unselected patient population 4 (9%) of 44 were scored as amplified [defined as multiple diffuse (orange-red) signals associated with one or more green signals within a nucleus, Fig. 3, *A* and *B*]. Of interest, 4 of 44 patients were also node positive at the time of initial diagnosis and in 3 of 4 node-positive patients, 8q24 amplification was observed.

Importantly, the clinical history of all patients was unknown to the laboratory performing the FISH experiments. Also, each case amplified for 8q24 was reevaluated to confirm the presence of amplified cells.

**CGH Analysis of Recurrent Prostate Tumor Tissue.** CGH was used to delineate the frequency of 8q copy number gain from three cell lines (du145, LNCap, and PC-3) and nine cases of recurrent prostate cancer. No copy number changes at 8q were found in either the du145 or LNCap cell lines. In contrast, the PC-3 cell line showed a gain of 8q13-qter and loss of 8pter-q12 (Fig. 3*C*).

The detailed CGH analysis of all chromosomal changes from the nine recurrent prostate cancers is presented elsewhere (20). This report focuses exclusively on the identification of increased copy number on 8q. In this study the increased copy number of 8q was found in 8 (89%) of 9 recurrent prostate cancers (Fig. 3*C*). Seven of these cases demonstrated a gain of the entire q arm. However, in one case, the gain was limited to 8q24-qter. Seven tumors also showed loss of 8p. Six tumors showed both 8p loss and 8q gain, with two demonstrating 8q gain and only one 8p loss.

## DISCUSSION

The work in this report further extends our knowledge of genetic changes of prostate cancer by identifying DNA sequence copy number changes of chromosome 8q as a marker of likely significance for the development of aggressive prostate cancers.

Utilization of chromosome microdissection provides a rapid approach to detect and clone amplified DNA sequences from cytologically recognizable markers such as dmin or hsrs (26). Other approaches to the analysis of amplified DNA sequences have relied on techniques based on DNA electrophoresis (*e.g.*, in gel renaturation or restriction landmark genomic scanning). While these techniques successfully identify amplified sequences, they are extremely laborious and can be confounded by amplified sequences unrelated to the phenotype of interest. By combining microdissection and FISH, this approach may be of practical importance since the methodology is available to perform rapid FISH pretreatment analysis on prostate biopsy core specimens that subsequently can be used for routine histopathological examination.

In this report 4 of 44 primary prostate cancers and 8 of 9 recurrent prostate tumors documented DNA copy number gains

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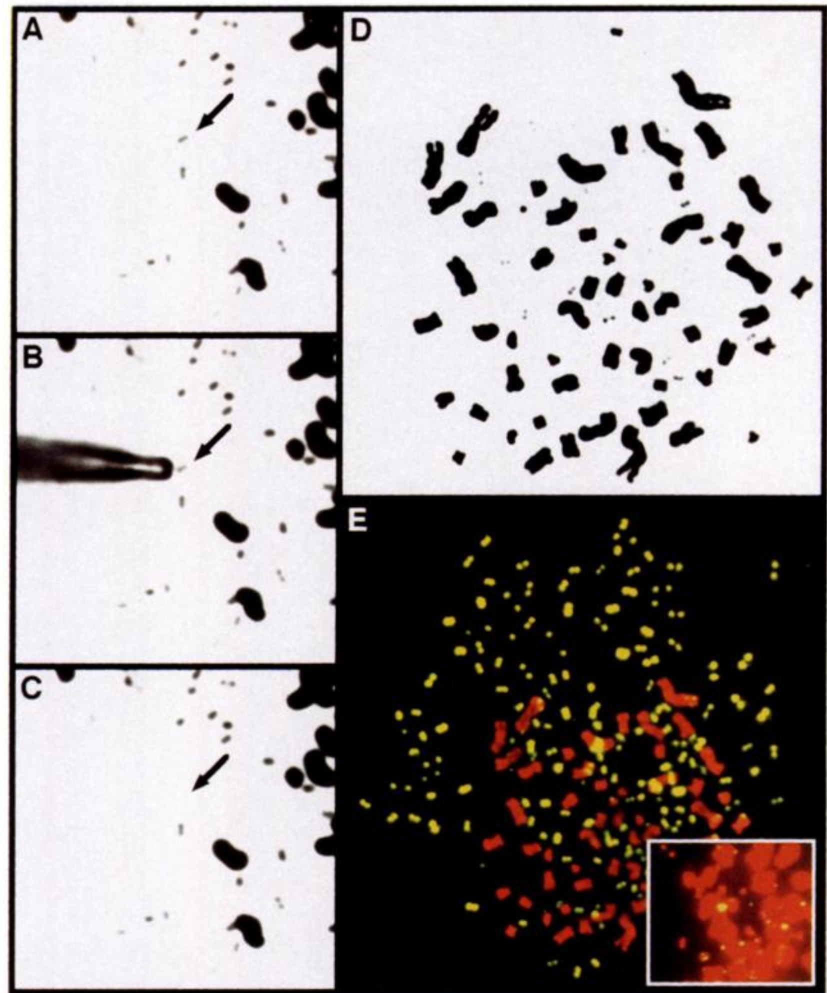


*Fig. 1* Utilization of chromosome microdissection to elucidate the chromosomal composition of dmin from a primary prostate cancer metaphase spread. In *A-C*, microdissection of a single dmin (*arrows*) from case PRO-39. In *D* and *E*, after microdissection of dmin (*A-C*), PCR amplification, and biotin labeling of the dissected DNA (see "Materials and Methods"), the PCR product was purified and hybridized by FISH back to the dmin to confirm that the dissection product hybridizes to the hsrs. The identical metaphase tumor cell from case PRO-39 is shown following Giemsa staining in *D* and following FISH in *E*. Note the FISH probe clearly identifies multiple dmin not recognized by conventional Giemsa staining. *Inset*, FISH of a biotin-labeled c-*myc* cosmid to metaphase cell from PRO-39, indicating hybridization to dmin (see text).

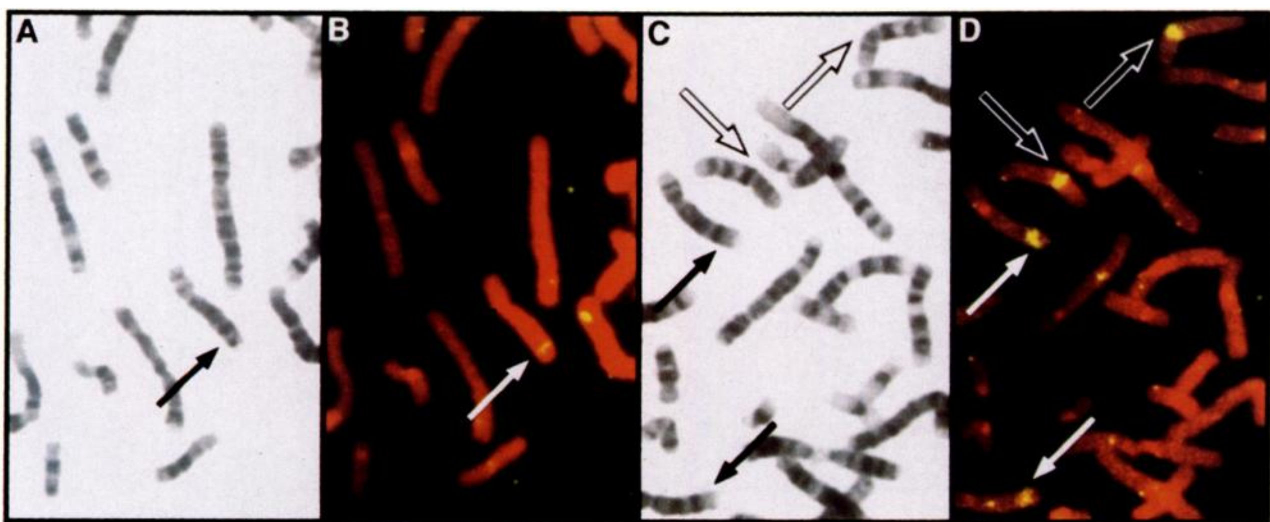


*Fig. 2* Chromosomal localization of amplified dmin DNA from the primary prostate cancer PRO-39 (*A* and *B*) and the prostate cancer cell line PC-3 (*C* and *D*) by FISH. *A*, G-banded normal partial metaphase with *arrow* to region of hybridization on chromosome 8; *B*, identical partial metaphase as in *A* following FISH identifying the normal chromosomal locus of the dissected dmin from PRO-39 as 8q24.1 (*arrow*); *C*, G-banded normal partial metaphase with *black arrows* to the region of hybridization on chromosome 8, and the *white arrows* to the region of hybridization on chromosome 10; *D*, identical partial metaphase as in *C* following FISH identifying the normal chromosomal locus of the dissected dmin from PC-3 as 8q24 and 10cen.

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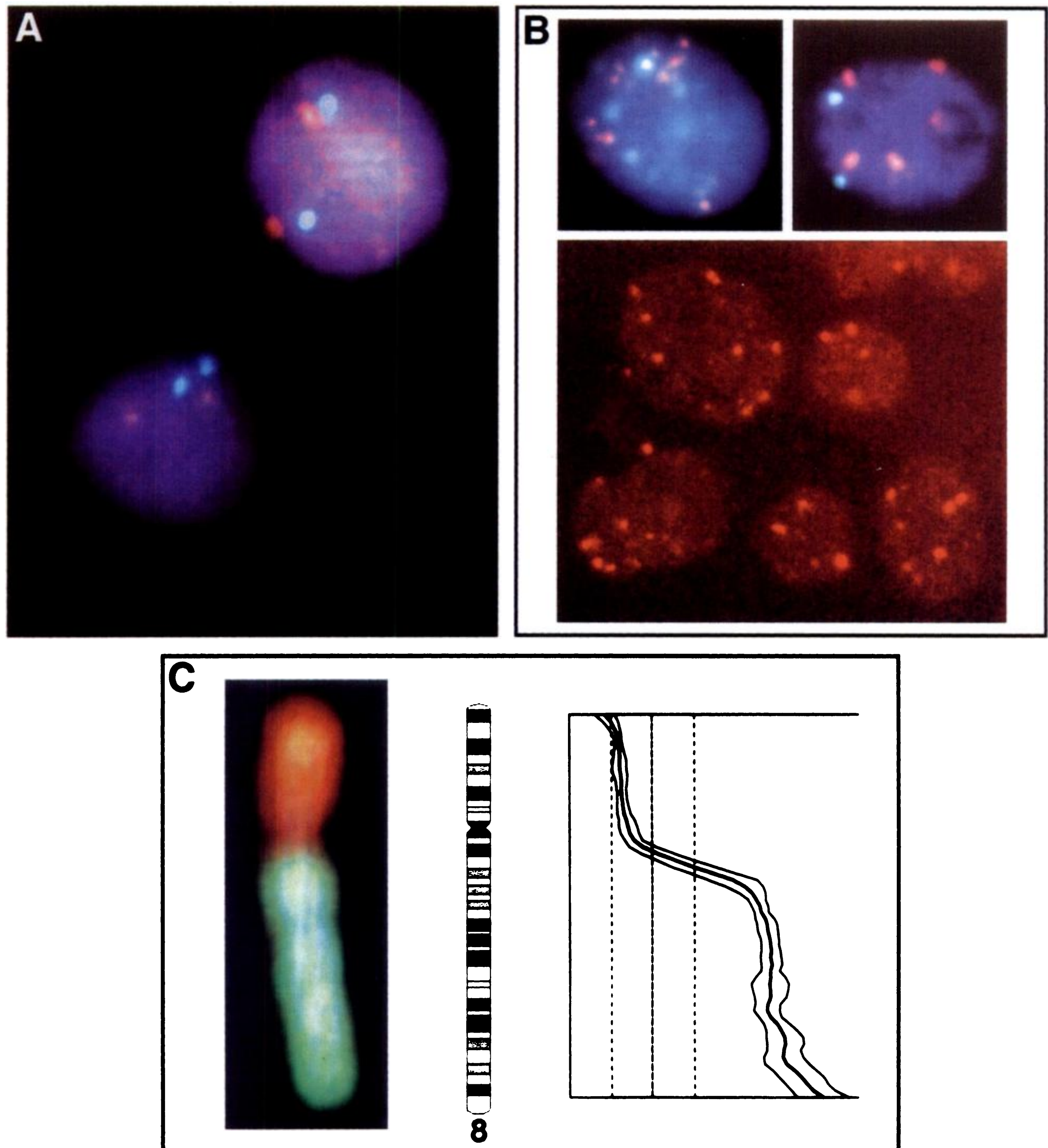


*Fig. 3* FISH (*A* and *B*) and CGH (*C*) analysis indicating DNA sequence copy number increase for 8q in primary (*B*) and recurrent (*C*) prostate cancer. *A*, FISH with a centromere probe for chromosome 8 (green) and a microdissection probe for 8q24 (isolated from PRO-39 dmin), on a touch preparation from normal prostate cells demonstrating two signals for each probe in interphase nuclei; *B*, *Upper panels*: FISH with a centromere probe for chromosome 8 (green) and the microdissection probe for 8q24 (red) to interphase nuclei from the amplified prostate case 33 (Table 1). Note that the prostate cancer cells show gains of 8q24 signals relative to the 8 centromere consistent with amplification of DNA sequences in this region. *Lower panel*: FISH using the microdissection probe for 8q24 (red) hybridized to interphase nuclei from the amplified prostate case 94–084 (Table 1), again demonstrating multiple copies of 8q24. *C*, CGH identifying DNA sequence copy number increase along the long arm of chromosome 8 (8q) from a case of recurrent prostate cancer.

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of 8q. For the primary tumors, a gain of chromosomal material at 8q24 was specifically identified by FISH, while in recurrent tumors CGH recognized 8q gain. By CGH whole-arm gain was observed in seven of nine tumors, with one case demonstrating amplification restricted to 8q24-qter. The commonly amplified region between all cancers was 8q24, and while in some tumors the degree of multiplication and size of the region involved may indicate a gross chromosomal change such as an isochromosome of 8q, clear documentation of gene amplification was observed in numerous cases. The data from both chromosome microdissection and CGH provides evidence for a common region of amplification spanning several megabases of DNA. The size of this segment is sufficiently large to contain several dozen genes and one or more of these may contribute to the growth advantage during development and reprogression of prostate tumors.

Of interest, the gain of 8q recognized by standard cytogenetic analysis has implicated its involvement in many other cancers including acute nonlymphocytic leukemia, hepatocellular carcinoma, renal cell cancer, gastric cancer, uveal melanoma, myelodysplastic syndrome, and colon cancer and may explain a broader role for 8q alteration in carcinogenesis. In this regard there is a strong candidate gene located within 8q24 which may be the target of the 8q amplification—c-*myc*. Although amplification of c-*myc* has not previously been reported in primary prostate malignancies, evidence for the overexpression of c-*myc* as a possible maker of a poor prognosis in prostate cancer has appeared previously (33). We directly demonstrated the presence of c-*myc* sequences within dmin from the PRO-39 tumor metaphases and the PC-3 and MPC-3 cell lines using FISH (Fig. 1*E*, *inset*). Although the c-*myc* gene is encoded within the dmin, previous estimation of the size of the c-*myc* amplicon (90–300 kilobases; Ref. 34) would account for $<<10\%$ of the total dmin DNA. Although it is likely that c-*myc* is the target of the amplification of 8q sequences, there may be other gene(s) involved in the apparent physiological effects of amplification of this chromosomal region.

Utilization of fresh tumor preparations for FISH has routinely allowed >500 nonoverlapping intact nuclei to be examined by FISH from the same biopsy specimen used for histological evaluation. The use of a microdissection probe for 8q24 provided FISH results equivalent to those for the more frequently studied centromeric probes. This study focusing on 8q is also in good general agreement with other earlier reports utilizing both FISH and CGH which clearly document chromosome 8 gain as a frequent change in prostate cancer progression (19, 20).

Our study illustrates the usefulness of chromosome microdissection in the analysis of dosage abnormality of chromosome changes in prostate cancers. We have identified a common region of DNA sequence amplification (8q24) and documented that it occurs in a subset of patients which may have a propensity for disease progression. We have further examined recurrent patient samples and shown an extremely high incidence of 8q gain, indicating this genetic alteration may result in a less favorable prognosis. While these data have to be taken in the context of the complex process of prostate carcinogenesis which involves not only gain but loss of chromosomal regions, if these preliminary results can be extended and confirmed the pretreatment detection of this cytogenetic marker of poor prognosis may be useful in selecting patients who would benefit from aggressive therapeutic intervention.

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