Original Article

Fluorescence In Situ Hybridization Aneuploidy as a Predictor of Clinical Disease Recurrence and Prostate-Specific Antigen Level 3 Years After Radical Prostatectomy

MAJ JAMES A. BROWN, USAF, MC; JEFFREY M. SLEZAK; MICHAEL M. LIEBER, MD; AND ROBERT B. JENKINS, MD, PHD

• *Objective*: To determine if fluorescence in situ hybridization (FISH) analysis of fresh-tissue biopsy specimens obtained at the time of radical prostatectomy is able to predict prospectively clinical disease progression or prostate-specific antigen (PSA) level in patients 3 to 4 years after surgery.

• *Materials and Methods*: FISH analysis was performed on fresh-tissue touch preparations obtained from 90 randomly selected radical prostatectomy specimens. Cut surface touch preparations from 40 specimens resected in 1992 were analyzed with DNA probes for chromosomes 4, 6-12, 17, 18, X, and Y. Needle-biopsy specimens were obtained from 50 tumors resected in 1993, and touch preparations from these specimens were studied with DNA probes for chromosomes 7, 8, 11, and 12. Serum PSA levels and clinicopathologic data were recorded, and each patient was followed up from the time of surgery to determine cancer progression.

• *Results*: Of 90 patients undergoing radical prostatectomy in 1992 and 1993, 89 returned for follow-up. Three patients received preoperative hormonal therapy, and in 2 patients, antiandrogen therapy was continued postoperatively. Fifteen patients underwent intraoperative orchiectomy immediately after radical prostatectomy, while 9 patients had postoperative adjuvant hormonal therapy. Six patients underwent postoperative radiation therapy. Fourteen patients (15.7%) demonstrated systemic, local, or PSA progression. Only 2 (4.7%) of 43 patients with FISH diploid tumors demonstrated cancer progression. Conversely, 10 (30.3%) of 33 FISH aneuploid and 12 (26.1%) of 46 FISH nondiploid tumors demonstrated cancer progression (P=.004 and P=.006, respectively). Unlike FISH, flow cytometric aneuploidy was not associated with early cancer progression. Elevated preoperative PSA concentration, increased preoperative and postoperative Gleason score, and increased preoperative and postoperative T or N stage were not statistically significantly associated with cancer progression. While chromosome 7 and 8 aneusomies were not statistically associated with cancer progression, 2 of 5 (P=.04) chromosome 12 aneusomic tumors demonstrated cancer progression.

• *Conclusion*: Early (within 4 years) local, systemic, or PSA progression occurred more frequently (P<.05) in radical prostatectomy patients with FISH aneuploid, nondiploid, and chromosome 12 aneusomic tumors. Flow cytometric ploidy status, preoperative serum PSA concentration, and clinical or pathologic grade or stage, including seminal vesicle involvement, margin status, and capsular perforation status, were not associated with early prostate cancer progression in this group of 89 patients. FISH analysis appears to be a useful preoperative tool for predicting aggressive vs indolent prostate cancer.

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FISH = fluorescence in situ hybridization; PIN = prostatic intraepithelial neoplasia; PSA = prostate-specific antigen

Although it has consistently been difficult to study prostate cancer using conventional cytogenetic techniques, we have found fluorescence in situ hybridization (FISH) analysis of prostate cancer biopsy specimens to be a useful tool in identifying chromosomal anomalies. In 5 previous studies, we asked the following questions: What numeric chromosomal anomalies are present in prostate cancer?[1] Is FISH a useful technique for studying needle-biopsy samples from prostate cancer specimens?[2] How do the chromosomal anomalies present in prostate cancer from patients with limited survival (<3 years) compare with the anomalies present in tumors from age-, grade-, and stage-matched control patients?[3] What chromosomal anomalies are present in high-grade (Mayo 3 or 4), high-stage (pT3 N0 M0) prostate tumors?[4] How do the chromosomal anomalies present in prostatic intraepithelial neoplasia (PIN), localized prostate cancer, and metastatic lymph nodes differ?[5]

These studies produced several important findings: (1) FISH is much more sensitive than flow cytometry for identifying aneuploidy (approximately 40% vs approximately

From the Department of Urology (J.A.B., M.M.L.), Section of Biostatistics (J.M.S.), and Department of Laboratory Medicine and Pathology (R.B.J.), Mayo Clinic Rochester, Rochester, Minn. Maj Brown is now with the 60th Medical Group, Travis AFB, Calif.

Address reprint requests and correspondence to Robert B. Jenkins, MD, PhD, Department of Laboratory Medicine and Pathology, Mayo Clinic Rochester, 200 First St SW, Rochester, MN 55905.

5%).[1] (2) Chromosomes 7 and 8 are the most common prostate cancer aneusomies identified to date.[1,2] (3) FISH aneuploidy and chromosome 7 and 8 aneusomy are associated with increased stage and grade.[1,2,4] (4) The rate of intratumoral ploidy heterogeneity in prostate cancer appears to be low (approximately 7%).[2] (5) Chromosome 7 and 8 aneusomies are observed in PIN.[2,5] (6) Unlike renal tumors, infiltrating leukocytes do not appear to be responsible for the chromosome 7 and 8 aneusomies present in prostate cancer.[1] (7) Chromosome 7, 8, and Y aneusomy, multiple chromosomal aneusomies, and hypertetraploidy are associated with clinical progression on retrospective analysis.[3] (8) Lymph node metastases have more chromosomal aneusomies than localized prostate cancer or PIN.[5]

Unfortunately, the prior association of FISH anomalies with prostate cancer progression has relied on retrospective analyses of patient cohorts. These cohorts received treatment over a long period when the means of detecting and treating prostate cancer were changing rapidly. Thus, the relevant question is this: Can FISH analysis of prostate cancer specimens obtained at the time of radical prostatectomy prospectively predict progression of clinical disease or serum PSA level? To answer this question, we followed up 89 patients who had radical prostatectomy with FISH analysis in 1992 and 1993 for a 4-year period to determine if FISH anomalies were associated with cancer progression.

MATERIALS AND METHODS

FISH Analysis

Detailed descriptions of the FISH materials and methods used in this study have been published previously.[1,2] Briefly, touch preparations from 40 sequential radical prostatectomies performed in 1992 were studied with DNA probes specific for the centromere region of 11 chromosomes (4, 6-12, 17, 18, and X) and for the middistal Yq (Yq12) region.[1] Touch preparations from 50 18-gauge needle-biopsy specimens taken from sequential radical prostatectomies performed in 1993 were studied with centromere-specific probes for chromosomes 7, 8, 11, and 12.[2] FISH DNA ploidy status was determined as described previously.[1,2]

Clinicopathologic Data

The patient's preoperative PSA level prior to neoadjuvant therapy, tumor grade, seminal vesicle involvement (if present), margins and capsular perforation status, and flow cytometric and FISH DNA ploidy status were recorded. Serum PSA concentration was measured with a monoclonal solid-phase, 2-site, immunoradiometric assay (Hybritech, Inc, San Diego, Calif). Flow cytometric analysis of formalin-fixed, paraffin-embedded prostatectomy specimens was performed as described previously.[6]

Postoperatively, patients were followed up at 3- to 4-month intervals for 2 years and then at approximately 6-month intervals. All follow-up examinations included digital rectal examination and PSA determination. Chest x-ray examination and radionuclide bone scan (with plain radiography when indicated) were obtained approximately once a year. In patients not returning to our institution, PSA concentration was determined at our clinic by a mailed blood specimen; alternatively, patients were contacted semiannually, and additional medical information was obtained from local physicians as indicated.[7] Dates of radiation and/or hormonal therapy (if applied) were recorded.

Eighty-nine of the 90 men returned to Mayo Clinic Rochester at least once during the 4 years of follow-up. Systemic prostate cancer progression and prostate cancer–specific death were used as clinical end points. Systemic progression was defined as clinical evidence of distant metastatic disease and ascertained by a rising PSA level or by positive findings on bone scan or other imaging tests. Prostate cancer–specific death was ascertained at the time of the patient's death by a combination of death certificate review, contact with the primary physician, and discussion with the patient's family, if necessary.

Statistical Analysis

Survival free of clinical disease or biochemical (PSA) progression was analyzed with use of the Kaplan-Meier method. Associations with survival were assessed with use of the log rank test. Multivariate analysis used the stepwise backward elimination procedure. All tests were 2 sided with a significance level of .05. The SAS system was used throughout.

RESULTS

Of 90 patients who had radical prostatectomy and FISH analysis of a portion of the surgical specimen in 1992 and 1993, 89 returned to Mayo Clinic for follow-up. Seventy-five patients (84.3%) demonstrated neither disease recurrence nor disease progression (Table 1). Thirteen patients (14.6%) demonstrated serum PSA progression of more than 0.2 ng/mL postoperatively. Three patients (3.4%) (2 with and 1 without serum PSA progression) had local recurrence, and 1 patient who had systemic recurrence died. Therefore, 14 patients (15.7%) demonstrated either systemic or local recurrence or serum PSA progression within 4 years after radical prostatectomy.

This group of 90 patients comprises 40 patients undergoing sequential radical prostatectomies in 1992[1] and a second group of 50 patients having the same surgery in 1993.[2] These 2 groups of sequential radical prostatectomy

Table 1. **4-Year Cancer Progression Rate Among 89 Patients After Radical Prostatectomy***

Status	No. (%) of patients
No progression	75 (84.3)
Systemic, local, or PSA progression	14 (15.7)
PSA progression (>0.2 ng/mL)	13 (14.6)
Systemic or local progression	4 (4.5)
Local progression	3 (3.4)
Systemic progression	1 (1.1)
Death	1 (1.1)

*PSA = prostate-specific antigen.

patients represented a typical cross-section of patients referred to our institution (Table 2). Twenty-three patients had a preoperative PSA level higher than 20 ng/mL, of whom 6 had a preoperative PSA level higher than 50 ng/mL. Thirty-two patients had a transrectal needle-biopsy specimen with a Gleason score of 7 or higher, of whom 13 had a Gleason score of 8 or higher. Thirty-nine patients had a radical prostatectomy specimen with a Gleason score of 7 or higher, of whom 5 had a prostatectomy specimen with a Gleason score of 8 or higher. Eighteen patients had clinical stage T3 (stage C) disease. Twenty-six patients had specimens demonstrating seminal vesicle involvement (pT3c). Forty-seven patients had positive margins (pT3), and 51 patients had a specimen that demonstrated cancer perforation of the capsule. Fourteen patients had node-positive disease.

Three patients received preoperative hormonal therapy. Of these, 1 had an orchiectomy at surgery and 1 received postoperative hormonal therapy. No patient had preoperative radiation therapy. Of the remaining 86 patients followed up, 15 underwent orchiectomy immediately after radical prostatectomy at the time of surgery. An additional 9 patients received postoperative adjuvant hormonal therapy, and another 6 patients received postoperative radiation.

FISH analysis identified 33 aneuploid tumors (36.7%) and 13 tetraploid tumors (14.4%). Conversely, flow cytometry identified 5 aneuploid tumors (5.6%) and 20 tetraploid tumors (22.2%). Ten (30.3%) of 33 FISH aneuploid and 12 (26.1%) of 46 FISH nondiploid tumors demonstrated cancer progression (Table 3). FISH aneuploidy and nondiploidy were associated with cancer progression to a

Table 2. **Clinicopathologic Data and 4-Year Cancer Progression Rate Among 89 Patients After Radical Prostatectomy***

Criteria	No. of patients	No. (%) progressing	*P* value†
Preoperative PSA (ng/mL)			
PSA >20	23	5 (21.7)	.35
PSA 20-50	17	4 (23.5)	.36
PSA >50	6	1 (16.7)	.86
Gleason score†			
TRNBx GS ≥7	32	7 (21.9)	.20
TRNBx GS ≥8	13	3 (23.1)	.43
TRNBx primary GS ≥4	20	4 (20.0)	.64
RRP GS ≥7	39	9 (23.1)	.07
RRP GS ≥8	5	1 (20.0)	.84
T stage			
Clinical T3	18	4 (22.2)	.46
SV involvement (pT3c)	26	6 (23.1)	.26
Positive margin (pT3)	47	7 (14.9)	.87
Capsular perforation (pT3)	51	9 (17.6)	.54
N stage			
≥1 Positive node	14	2 (14.3)	.90
≥2 Positive nodes	4	0 (0)	.39
≥3 Positive nodes	3	0 (0)	.46
Adjuvant therapy			
Preoperative/intraoperative hormones	17	3 (17.6)	.44
Postoperative hormones	9	1 (11.1)	.64
Postoperative radiation	6	0 (0)	.29

*GS = Gleason score; PSA = prostate-specific antigen; RRP = radical retropubic prostatectomy; SV = seminal vesicle; TRNBx = preoperative transrectal needle biopsy.

†Compared with patients with tumors lacking the given criteria.

highly significant level (P=.004 and P=.006, respectively). Conversely, 0 of 5 flow cytometric aneuploid and 5 of 25 flow cytometric nondiploid tumors demonstrated cancer progression (P=.36 and P=.45, respectively).

Chromosomes 7, 8, and 12 were the most common chromosomal aneusomies identified. Two of 5 patients with tumors demonstrating chromosome 12 aneusomy have demonstrated cancer progression (P=.04) (Table 4). Although 30 tumors demonstrated chromosome 7 and/or chromosome 8 aneusomy, only 7 of these tumors demonstrated cancer progression (P=.18). Similarly, only 3 of 21 tumors with gain of chromosome 7 have demonstrated cancer progression (P=.84). Two of 5 tumors with chromosome 8 loss developed cancer progression (P=.15), while only 3 of 15 tumors with chromosome 8 gain demonstrated cancer progression to date (P=.64).

We analyzed other clinicopathologic factors (preoperative serum PSA level; pathologic grade [Gleason score]; pathologic stage, including seminal vesicle involvement, margin status, and capsular perforation status) to determine if any other statistically significant association with cancer progression was present (Table 2). None was observed.

Seventeen patients received antiandrogen therapy preoperatively or intraoperatively (orchiectomy or bilateral orchiectomy immediately after radical prostatectomy) as treatment for stage pT3 or node-positive disease. Only 3 of these patients have shown cancer progression to date (P=.44).

Only 1 of 9 patients who had antiandrogen therapy initiated postoperatively has demonstrated subsequent cancer progression to date (P=.64) (Table 2).

Six patients had radiation therapy approximately 3 months after radical prostatectomy as treatment for stage pT3 disease. One patient had an elevated PSA (0.8 ng/mL) at the time that radiation therapy was initiated. No patient receiving radiation has demonstrated further clinical disease or serum PSA progression (P=.29). A multivariate analysis was performed using a Cox model with the variables FISH nondiploidy, Gleason score, preoperative PSA, seminal vesicle involvement, and extracapsular extension. At all steps in the backward variable elimination procedure only FISH nondiploidy independently predicted early prostate cancer progression (P=.02).

DISCUSSION

Prostate cancer is, arguably, the most important tumor in urologic oncology, and its commonness combined with its widely varying aggressiveness makes it one of the most important subjects of oncologic research.

Pathologic stage and histologic grade are the traditional methods of predicting a tumor's malignant potential. Numerous investigators have reported that grade[8-12] and stage[12,13] are associated with cancer progression. Others have found that stage[14-17] and grade[8,15,16,18-22] are further associated with decreased survival. Seminal vesicle involvement,[23,24] positive margin status,[7,10,25] and tumor volume[26,27] have all been associated with cancer progression. Conversely, other authors have questioned whether stage[17,28-30] or grade[14,28-31] is associated with either cancer progression or decreased survival, and capsular penetration has been reported not to correlate with cancer progression.[25] Therefore, even though grade and stage predict cancer progression or worsening survival for a group of patients, neither criterion is sensitive enough to predict the outcome for a given patient. Further, while pretreatment serum PSA level has been identified as a prognostic factor,[32-36] preoperative serum PSA level has also been reported not to correlate with cancer progression.[37,38]

During the last decade, flow cytometry has been used to determine DNA ploidy as well as S-phase fraction in an effort to provide additional prognostic information. Nu-

Table 3. **DNA Ploidy Status and 4-Year Cancer Progression Rate Among 89 Patients After Radical Prostatectomy***

Criteria	No. of patients	No. (%) progressing	P value
FISH diploid	43	2 (4.7)	.99
FISH nondiploid†	46	12 (26.1)	.006‡
FISH aneuploid	33	10 (30.3)	.004§
FCM diploid	64	9 (14.1)	.55
FCM nondiploid†	25	5 (20.0)	.45‡
FCM aneuploid	5	0 (0)	.36§

*FCM = flow cytometry; FISH = fluorescence in situ hybridization.
†Nondiploid includes both aneuploid and tetraploid tumors.
‡Compared with diploid tumors.
§Compared with nonaneuploid tumors.

Table 4. **Specific Chromosome Aneusomy and 4-Year Cancer Progression Rate Among 89 Patients After Radical Prostatectomy**

Chromosomal criteria	No. of patients	No. (%) progressing	P value*
12 Aneusomy	5	2 (40.0)	.04
7 Gain and/or 8 aneusomy	30	7 (23.3)	.18
7 Gain†	21	3 (14.3)	.84
8 Aneusomy	20	5 (25.0)	.23
8 Gain	15	3 (20.0)	.64
8 Loss	5	2 (40.0)	.15

*Compared with nonaneusomy loss or gain of the specified criteria.
†No tumor demonstrated loss of chromosome 7.

merous authors have reported that flow cytometric nondiploidy is associated with cancer progression[20,23,24,28,30,39-44] as well as decreased survival.[8,19-21,29,30,40,41,45-47] S-phase fraction has shown additional prognostic information including correlation with cancer progression and decreased survival.[8,18,20,29,42,48] Conversely, other investigators have reported that flow cytometric ploidy status is not associated with cancer progression[49] or decreased survival.[14,16,18,31,50]

Lifson et al[51] retrospectively studied 15 paraffin-embedded radical prostatectomy specimens with FISH using DNA probes for chromosome 7 and 10 centromeres. They reported a 56% clinical progression rate for FISH hyperdiploid tumors compared with a 17% progression rate for FISH diploid carcinomas.

We have performed several previous studies using FISH to analyze prostate cancer specimens.[1-5] Several groups have demonstrated that FISH is more sensitive than flow cytometry for identifying aneuploidy.[1,51] A retrospective analysis of paraffin-embedded tissues demonstrated that chromosome 7, 8, and Y aneusomy, as well as multiple chromosomal aneusomies and hypertetraploidy, are associated with cancer progression and decreased survival.[3,4] To our knowledge, this is the first study to use FISH to analyze prostate cancer specimens at the time of surgery in patients followed up prospectively to determine disease progression.

In 40 specimens, FISH analysis was performed on touch preparations taken from the fresh cut tumor surface.[1] In 50 specimens, FISH analysis was performed on touch preparations taken from 18-gauge needle-biopsy specimens from visible tumor foci observed in the radical prostatectomy specimens at the time of surgery.[2] All 90 prostatectomy specimens were sequential, randomly selected cases that demonstrated the typical distribution of pathologic grade and stage seen at the Mayo Clinic in the 1990s. Twenty-six of these patients received adjuvant hormonal therapy, and 6 patients received adjuvant radiation therapy. This large number of patients receiving adjuvant hormonal and radiation therapy is notable for several reasons. While we are unaware of any report of preoperative hormonal therapy affecting FISH or flow cytometry ploidy or causing chromosome alterations, preoperative hormonal therapy may have affected the pathologic findings of the radical prostatectomy specimens in the 3 patients so treated. Clearly adjuvant hormonal or radiation therapy likely affected the cancer progression rates of the 32 patients so treated, and adjuvant therapy is likely the primary reason that preoperative and postoperative grade and stage did not correlate with cancer progression.

Thus, while FISH analysis provided novel information in regard to cancer progression in this group of patients, many of whom were treated with adjuvant hormonal and radiation therapy, this may not be the case for a group of radical prostatectomy patients not receiving adjuvant hormonal or radiation therapy. It is interesting that FISH aneuploidy and nondiploidy were still able to predict prospectively early cancer progression in this group of patients despite considerable use of hormonal and radiation therapy. Only 2 of the 40 tumors demonstrating cancer progression were from tumors classified as FISH diploid. Use of a greater number of DNA probes might have resulted in classifying these tumors as DNA aneuploid.

Acquisition of follow-up information or of patient return was not linked to FISH ploidy or aneusomy results. There is no reason to expect that the extent and degree of follow-up would be any different in the FISH aneuploid or nondiploid groups compared with the FISH diploid groups.

As only 3 patients received hormonal therapy prior to radical prostatectomy (the remainder underwent orchiectomy immediately after pelvic lymph node dissection and radical prostatectomy or had hormonal or radiation therapy initiated postoperatively), preoperative (transrectal needle biopsy) and postoperative (radical prostatectomy) grade and stage as well as preoperative PSA level (which was determined before any treatment) should have been valid in 86 of the 89 patients. Thus, while hormonal and radiation therapy likely decreased cancer progression and likely affected the prognostic accuracy of grade and stage, adjuvant therapy did not eliminate the ability of FISH to predict cancer progression in this group of patients. FISH ploidy classification was clearly the most sensitive criterion for predicting early cancer progression.

The observation that chromosome 12 aneusomy was associated with early cancer progression while chromosome 7 and 8 aneusomy was not may suggest that additional chromosomal alterations beyond chromosome 7 and 8 aneusomy are required for early (within 4 years) cancer progression. However, had only 1, rather than 2, of the 5 chromosome 12 aneusomy patients had disease progression, chromosome 12 aneusomy would not have reached statistical significance. A larger number of patients followed up for a longer time may have demonstrated that chromosome 7 and 8 aneusomies do, in fact, correlate with cancer progression.

In summary, FISH analysis (aneuploidy or nondiploidy) prospectively determined tumors at greater risk for early (within 4 years) cancer progression in a group of patients, many of whom received adjuvant hormonal or radiation therapy. Furthermore, the other currently used techniques for identifying aggressive prostate cancer (elevated preoperative serum PSA concentration, high Gleason score, capsular perforation, margin positivity, seminal vesicle involvement, positive lymph node status, and flow cytometry

ploidy status) did not correlate to a statistically significant degree with early cancer progression ($P<.05$) in this group of patients.

FISH analysis remains an experimental technique, and efforts to mechanize tumor analysis using FISH are ongoing. If a successful and reproducible technique for mechanizing FISH analysis is developed and further studies comparing FISH and clinical parameters in patients undergoing radical prostatectomy with and without adjuvant hormonal or radiation therapy confirm those results, FISH analysis may become a promising preoperative tool for predicting aggressive vs nonaggressive prostate cancer.

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