8q Gain Is an Independent Predictor of Poor Survival in Diagnostic Needle Biopsies from Prostate Cancer Suspects

Franclim R. Ribeiro,[1,7] Carmen Jerónimo,[1,5] Rui Henrique,[2,6] Daniel Fonseca,[3] Jorge Oliveira,[4] Ragnhild A. Lothe,[7,8] and Manuel R. Teixeira[1,6]

Abstract

Purpose: The main procedure to confirm a suspected diagnosis of prostate cancer is histologic analysis of ultrasound-guided sextant prostate biopsies. As it is difficult to reliably assess tumor stage and grade in such minute samples, the clinical significance of some tumor foci remains unclear. Genetic markers that could augment pretreatment prognostic information would improve the clinical management of the disease.

Experimental Design: We have analyzed by comparative genomic hybridization a consecutive series of prostate needle biopsies obtained prospectively from 100 prostate cancer suspects. For 25 of these patients, a second independent biopsy core was analyzed to assess possible tumor heterogeneity. Additionally, a three-color fluorescent *in situ* hybridization assay was done in paraffin-embedded biopsy cores to validate the comparative genomic hybridization findings and to confirm their prognostic value.

Results: Sixty-one of 100 biopsy samples had morphologic evidence of prostate cancer and 41 (67%) of these displayed genomic copy number changes as opposed to none of the morphologically normal biopsies. The presence of losses, amplifications, and the total number of genomic imbalances were significantly associated with poorly differentiated tumors. Kaplan-Meier curves with log-rank test showed that patients whose tumors displayed 8q gains had a significantly worse survival even when tumor grade was taken into account ($P = 0.008$). Restricting the analysis to cases with Gleason score 7, the most troublesome category in terms of prognostic information, gains at 8q were still significantly associated with poor survival ($P = 0.011$), something that was confirmed by fluorescent *in situ* hybridization in an independent series of biopsies with much longer follow-up time ($P = 0.023$).

Conclusions: We show that whole genomic information can be obtained from minute needle biopsies of prostate cancer suspects and that genetic data can provide additional prognostic information before a therapeutic decision is taken.

Confirmation of a suspected diagnosis of prostate cancer is usually obtained by histologic analysis of ultrasound-guided sextant biopsies of the prostate (1, 2). Morphologic assessment of tumor grade and extent in these minute samples suffers from poor reproducibility, which can be partly explained by sample representativeness and the pathologist's experience (3). Large retrospective studies comparing the findings in matched biopsy and prostatectomy specimens have shown that up to 57% of the tumors are downgraded and ~20% are overgraded at biopsy when compared with their prostatectomy counterparts (4–12), further indicating that small, well-differentiated tumors are more often misclassified. With the widespread use of prostate-specific antigen (PSA) screening resulting in the detection of an increasing proportion of these low- and intermediate-grade prostate carcinomas, the clinical significance of which is often difficult to ascertain, it is expected that molecular markers with prognostic value in biopsy samples would contribute to better-informed therapeutic decisions for this clinically heterogeneous disease.

Most of the genetic information available on prostate cancer was obtained through the analysis of prostatectomy samples, resulting in a bias toward lower-staged cancers for which this therapeutic option is usually offered (13–16). Patients presenting extraprostatic disease are not eligible for surgical treatment; thus, genetic knowledge on this aggressive subtype of prostate cancer is more limited (17–20). Some investigators have tried to obtain biological information on prostate cancer by assessing diagnostic needle biopsies using DNA ploidy analysis

Authors' Affiliations: Departments of [1]Genetics, [2]Pathology, [3]Radiology, and [4]Urology, Portuguese Oncology Institute-Porto; [5]Fernando Pessoa University; [6]Department of Pathology and Molecular Immunology, Institute of Biomedical Sciences, University of Porto, Porto, Portugal; [7]Department of Genetics, Institute for Cancer Research, Norwegian Radium Hospital; and [8]Department of Molecular Biosciences, University of Oslo, Oslo, Norway

Received 9/8/05; revised 2/20/06; accepted 4/27/06.

Grant support: Fundação para a Ciência e a Tecnologia grant POCTI/CBO/38853/2001, Norwegian Cancer Society grant A95068 (R.A. Lothe), and Fundação para a Ciência e a Tecnologia grant SFRH/BD7067/2001 (F.R. Ribeiro).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Manuel R. Teixeira, Department of Genetics, Portuguese Oncology Institute, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal. Phone: 351-225084000; Fax: 351-225084016; E-mail: mteixeir@ipoporto.min-saude.pt.

©2006 American Association for Cancer Research.

doi:10.1158/1078-0432.CCR-05-1977

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/12/13/3961/1963238/3961.pdf by guest on 09 December 2023

Table 1. Clinicopathologic data and corresponding genetic findings in 125 biopsy samples from 100 prostate cancer suspects

ID*	Age	PSA (ng/mL)	Tumor content (%)†	Gleason score	Clinical stage	Treatment‡	Follow-up status§	CGH findings (99% standard reference interval)
6	65	22.00	Neg	—				No changes
9	65	11.00	Neg	—				No changes
25	49	11.40	Neg	—				No changes
31	70	13.00	Neg	—				No changes
33	58	8.57	Neg	—				No changes
37	71	11.50	Neg	—				No changes
48	69	10.10	Neg	—				No changes
51	71	7.12	Neg	—				No changes
53	58	7.94	Neg	—				No changes
55	82	16.40	Neg	—				No changes
58	69	8.84	Neg	—				No changes
64	65	9.00	Neg	—				No changes
70	63	5.48	Neg	—				No changes
76	53	7.24	Neg	—				No changes
80	50	16.60	Neg	—				No changes
81	66	9.84	Neg	—				No changes
83	72	14.40	Neg	—				No changes
84	68	9.22	Neg	—				No changes
88	78	9.32	Neg	—				No changes
89	64	6.60	Neg	—				No changes
90	58	5.05	Neg	—				No changes
91	79	25.00	Neg	—				No changes
92	66	11.50	Neg	—				No changes
94	66	7.34	Neg	—				No changes
95	61	9.08	Neg	—				No changes
99	67	5.90	Neg	—				No changes
100	65	29.40	Neg	—				No changes
17a	68	8.50	Neg	—				No changes
18	76	10.10	N	6 (3 + 3)	II	ADT		No changes
19	62	9.14	N	6 (3 + 3)	II	PT		No changes
35	65	14.00	N	6 (3 + 3)	II	RT		No changes
39	71	11.80	N	6 (3 + 3)	II	RT + ADT		No changes
47	61	8.80	N	6 (3 + 3)	II	PT		No changes
78	69	109.30	N	6 (3 + 3)	IV	ADT		No changes
79	64	14.78	N	6 (3 + 3)	II	PT		No changes
98	62	27.41	N	6 (3 + 3)	II	RT + ADT		No changes
3a	75	17.75	0-25	6 (3 + 3)	II	RT		No changes
3b			0-25					No changes
5	51	4.52	50-75	6 (3 + 3)	II	PT		rev ish enh(8q23q24),dim(8p12p22)
40	80	15.80	0-25	6 (3 + 3)	IV	ADT		No changes
93	63	14.00	0-25	6 (3 + 3)	II	ADT		No changes
15a	73	7.96	0-25	6 (3 + 3)	II	ADT	Dec	No changes
15b			0-25					No changes
14	79	62.60	75-100	6 (3 + 3)	II	ADT		No changes
56	73	12.37	75-100	6 (3 + 3)	II	RT + ADT		No changes
61	67	14.20	50-75	6 (3 + 3)	II	PT		No changes
62	75	10.70	50-75	6 (3 + 3)	II	ADT		No changes
65	71	30.80	75-100	6 (3 + 3)	II	ADT		rev ish dim(8p12pter,13q14q31,16q21qter)
12a	68	24.00	75-100	6 (3 + 3)	II	RT + ADT		No changes
12b			75-100					rev ish dim(6q16q22)
13a	73	15.72	75-100	6 (3 + 3)	IV	ADT	Dec	rev ish enh(5p15,7p,7q21qter,20p12pter,20q13q13), dim(5q13q31,6q14q22,8p21,13q14q31)

(*Continued on the following page*)

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/12/13/3961/1963238/3961.pdf by guest on 09 December 2023

Table 1. Clinicopathologic data and corresponding genetic findings in 125 biopsy samples from 100 prostate cancer suspects (Cont'd)

ID*	Age	PSA (ng/mL)	Tumor content (%)†	Gleason score	Clinical stage	Treatment‡	Follow-up status§	CGH findings (99% standard reference interval)
13b			75-100					rev ish dim(6q15q23,8p12p21)
21a	67	14.50	75-100	6 (3 + 3)	II	RT + ADT		rev ish dim(8p21p22)
21b			25-50					No changes
41a	77	14.60	50-75	6 (3 + 3)	II	ADT		No changes
41b			0-25					No changes
45a	68	30.60	75-100	6 (3 + 3)	IV	ADT		rev ish enh(3q23q24,3q26q28)
45b			0-25					No changes
57a	60	11.40	75-100	6 (3 + 3)	II	PT		rev ish enh(3q23q28,20p13)
57b			0-25					No changes
69a	80	287.01	25-50	6 (3 + 3)	IV	ADT		rev ish dim(8p12p23)
69b			75-100					rev ish dim(8p12p22,14q24q32)
85a	69	46.50	75-100	6 (3 + 3)	II	ADT		No changes
85b			25-50					No changes
34	71	8.31	0	7 (3 + 4)	II	PT		No changes
46	78	89.30	75-100	7 (3 + 4)	II	ADT		rev ish dim(8p22p23,16q23q24)
59	70	43.00	25-50	7 (3 + 4)	II	ADT		No changes
87a	67	40.60	0-25	7 (3 + 4)	III	ADT		rev ish dim(8p12p23)
87b			0					No changes
4	66	7.00	75-100	7 (3 + 4)	II	PT	Rec	rev ish enh(8q),dim(2q21q31,6q13q16, 8p,16q21qter,18q12q21)
7	69	13.60	50-75	7 (3 + 4)	III	RT + ADT		rev ish dim(10q23q24)
11	64	52.10	75-100	7 (3 + 4)	IV	ADT	Rec	rev ish enh(3p13p14,3p25pter,3q13q22)
16	67	11.40	50-75	7 (3 + 4)	II	PT		rev ish enh(3p23pter,3q25q28,8q21qter), dim(8p12p22,10q21q24,17p13)
30	62	21.47	50-75	7 (3 + 4)	II	RT + ADT		rev ish enh(3p14p26,3q13q28,5p,5q21q35,7p13p15, 7q11q36,8q11q24,16p,18p11),dim(8p12p22,18q12q23)
42	70	8.56	75-100	7 (3 + 4)	II	RT + ADT		rev ish dim(8p12p22)
44	72	17.50	75-100	7 (3 + 4)	III	RT + ADT		rev ish enh(1q22q23,10q21q22,18p11),dim(1q41qter, 8p21p22,10q24q25,18q12q22)
52	70	45.00	75-100	7 (3 + 4)	II	ADT		No changes
72	73	89.46	75-100	7 (3 + 4)	IV	ADT		rev ish enh(8q21qter),dim(8p12pter,10q22q23,12p, 16q23qter,17p12pter,22q21q22)
10a	78	22.60	75-100	7 (3 + 4)	II	ADT	Dec	rev ish dim(8p12p22)
10b			0-25					No changes
26a	66	18.54	75-100	7 (3 + 4)	II	ADT	Dec	rev ish enh(5p13pter,7,8q,17p11p12),dim(5q11q13, 8p,13q14q21,17p13),amp(8q,17p11p12)
26b			50-75					rev ish enh(5p13p15,7p13p21,7q31q35,8q,13q12, 13q32q34,17p11p12),dim(5q11q13,8p12p23,13q21)
27a	61	136.00	50-75	7 (3 + 4)	III	ADT		No changes
27b			0-25					No changes
60a	62	12.60	75-100	7 (3 + 4)	II	PT		rev ish enh(3q13qter),dim(8p22p23)
60b			75-100					rev ish enh(3q26q27),dim(8p21p22)
2	73	63.10	0	7 (4 + 3)	III	ADT	Dec	No changes
50	60	64.50	50-75	7 (4 + 3)	IV	ADT		rev ish enh(7,8q13qter),dim(5q22q31, 6q13q21,8p21p23,16q22q23)
63	70	64.00	25-50	7 (4 + 3)	III	ADT		rev ish enh(Xp22,1q22q32,5p14pter,8p11p12,8q), dim(8p21pter,10p12pter,12p13,13q13q14,14q11q12, 16q23qter),amp(Xp22,8p11p12,8q22qter)
67	69	5.30	50-75	7 (4 + 3)	II	RT		No changes
8	71	11.60	25-50	7 (4 + 3)	II	RT + ADT	Rec	rev ish enh(8q12q24),dim(2q21q23, 6q16q22,12p12,13q14)
20	68	17.90	75-100	7 (4 + 3)	II	RT + ADT		rev ish dim(13q21q22)

(*Continued on the following page*)

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/12/13/3961/1963238/3961.pdf by guest on 09 December 2023

Table 1. Clinicopathologic data and corresponding genetic findings in 125 biopsy samples from 100 prostate cancer suspects (Cont'd)

ID*	Age	PSA (ng/mL)	Tumor content (%)†	Gleason score	Clinical stage	Treatment‡	Follow-up status§	CGH findings (99% standard reference interval)
22	68	36.30	75-100	7 (4 + 3)	III	RT + ADT		rev ish enh(7p15pter,7q21qter),dim(5q14q31, 6q13q22,8p12p22,13q12q22)
49	67	7.35	75-100	7 (4 + 3)	III	RT + ADT		rev ish enh(7q31,8p12qter,17q24q25),dim(5q11q21,8p22pter, 10q22q24,13q,16q,17p13),amp(8p12qter)
66	76	130.00	50-75	7 (4 + 3)	III	ADT		rev ish enh(3q13qter,4q21q22,7q31q36),dim(1p22p31, 5q23,6q16q22,13q14,15q21),amp(4q21q22)
75	76	40.59	50-75	7 (4 + 3)	IV	ADT		rev ish dim(5q21q23,6q14q16)
96	65	7.29	50-75	7 (4 + 3)	II	RT + ADT		No changes
1a	77	52.00	75-100	7 (4 + 3)	II	ADT	Dec	rev ish enh(8q),dim(2q11q12,8p,16q),amp(8q)
1b			75-100					rev ish enh(8q),dim(8p12p22,16q,18q12qter)
23a	69	9.55	75-100	7 (4 + 3)	II	PT		rev ish enh(8q21q23),dim(8p12p22)
23b			75-100					rev ish dim(8p21p22)
54a	78	55.50	75-100	7 (4 + 3)	II	ADT		rev ish enh(5p15,7q31,10q21,21q22),dim(1p21p31, 5q11q13,6q16q22,8p12p22),amp(10q21)
54b			75-100					rev ish enh(1q31),dim(5q13q21,6q21q22,8p21p22)
77a	66	42.68	50-75	7 (4 + 3)	II	RT + ADT		rev ish dim(5q12q13,8p,13q21q31,14q24q31)
77b			50-75					rev ish dim(8p21p22,13q21)
82a	86	158.00	75-100	7 (4 + 3)	IV	ADT	Dec	rev ish enh(1p12p31,1q,3q21q26,7,8q,17q22qter), dim(3q28qter,6q15q23,8p12p22,9q21)
82b			75-100					rev ish enh(1q31q41,3q21q26,8q11q12, 8q21q23,8q24),dim(6q16q22,8p21)
97a	86	17.40	75-100	7 (4 + 3)	III	ADT		rev ish enh(3q21q22,7,17q21q24),dim(2q14q24, 6q24,8p12p22,13q14q34,16q21qter)
97b			0-25					No changes
28	56	500.00	50-75	8 (3 + 5)	IV	ADT		rev ish enh(1q,6q23q26,7q11q22,8q13qter, 11q13q23,16p12,17q23q25),dim(8p12p23, 13q21q31,16q),amp(6q23q25,7q11q22,8q21qter)
24	80	19.20	50-75	8 (4 + 4)	III	ADT		rev ish dim(6p21)
32	80	570.00	50-75	8 (4 + 4)	IV	ADT		No changes
38	73	8.17	25-50	8 (4 + 4)	III	RT + ADT		rev ish dim(5q11q13,5q21q23,8p,10q22q25,12q22q23,17p)
43	75	77.90	50-75	8 (4 + 4)	III	ADT	Dec	rev ish dim(8p12p23,16q23)
71	71	11.02	75-100	8 (4 + 4)	II	PT		No changes
73	64	10.90	75-100	8 (4 + 4)	II	RT + ADT		rev ish enh(8q13qter),dim(13q14q31),amp(8q22q23)
29a	65	542.00	75-100	8 (4 + 4)	IV	ADT		rev ish enh(3p12,3q21q26),dim(8p12p23),amp(3q26)
29b			50-75					rev ish enh(3p12p13,3q13q26),dim(6q16q22,8p12p23, 11q23qter,13q14q22,16q),amp(3p12,3q22q26)
86	80	9.36	0	9 (4 + 5)	IV	ADT		No changes
68a	85	48.60	0-25	9 (4 + 5)	II	ADT		No changes
68b			50-75					No changes
74a	57	520.00	25-50	9 (4 + 5)	IV	ADT	Dec	rev ish enh(1q,8q12qter,9q21q22,9q33qter),dim(8p12p22)
74b			25-50					rev ish enh(1q,8q13qter),dim(8p21)
36a	66	154.00	50-75	9 (4 + 5)	IV	ADT		No changes
36b			25-50					No changes

*Samples labeled with a and b represent two spatially distant research cores analyzed independently in 25 prostate cancer patients to assess tumor heterogeneity.
†Estimated tumor content on morphologic analysis: Neg, no tumor detected in routine nor research cores; N, tumor detected in routine cores but not in the corresponding research cores.
‡Treatment: ADT, androgen deprivation therapy; RT, radiotherapy; PT, prostatectomy.
§Follow-up status: Dec, deceased; Rec, clinical recurrence.

(21, 22), fluorescent *in situ* hybridization (FISH) with selected centromeric (23–26) and/or locus-specific (27, 28) probes, and, more recently, methylation analysis and expression studies of candidate genes (29–32). However, most groups used retrospectively selected, paraffin-embedded biopsy cores, thus facing inherent technical and methodologic limitations. In the particular case of FISH analysis, it is difficult to reliably assess losses of genetic material, the most common type of genetic change in

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/12/13/3961/1963238/3961.pdf by guest on 09 December 2023

prostate cancer, in archival interphase cells. Furthermore, even if gains can more easily be scored, several chromosomal regions not usually selected for analysis are also frequently involved in prostate carcinogenesis, indicating that previous FISH studies may have overlooked important genetic events.

We have recently shown that it is possible to obtain whole genome information on fresh-frozen needle biopsies from prostate cancer patients (33). Following up on that pilot study, we now address the potential prognostic effect of genomic imbalances in a prospective series of sextant biopsies obtained from 100 prostate cancer suspects being consecutively evaluated at our institution. The comparative genomic hybridization (CGH) technology and our study design ensure unbiased genetic information from a series of samples expected to represent all stages of prostate cancer progression as well as nonmalignant disease. Additionally, a second biopsy core was analyzed for a subgroup of the patients to assess possible tumor heterogeneity.

Materials and Methods

Biopsy specimens. One hundred individuals referred to our institution due to elevated PSA levels were enrolled in this study after informed consent. They followed the standard clinical evaluation procedures in use at the Portuguese Oncology Institute (Porto, Portugal) and were subsequently submitted to ultrasound-guided sextant prostate biopsies. In addition to the standard six cores obtained by this method, two supplementary tissue samples (paired with two of the former group) were collected from the more suspicious areas and frozen at −80°C to be used for research purposes alone. The diagnostic cores were formalin-fixed and paraffin-embedded for standard histopathologic analysis, and tumor grade was determined according to Gleason score (34). The percentage of cores with evidence of cancer, age, PSA levels, and pretreatment clinical stage were registered for each patient. Whenever prostate carcinoma was diagnosed, patients followed the corresponding staging and treatment procedures, which comprised radical prostatectomy, radiotherapy, or androgen deprivation therapy. Only the frozen cores were used for genomic analysis. Five-micron sections of these samples were cut, stained, and evaluated by a pathologist to assess the presence and proportion of neoplastic tissue, after which the whole core was sectioned and processed for DNA extraction. Initially, only one randomly selected research core per patient was assessed for DNA copy number changes. Afterward, the second biopsy core with morphologic evidence of tumor was analyzed in 25 of the patients to assess tumor heterogeneity (these additional cores were collected from opposite prostatic peripheral lobes).

Validation of copy number changes was done by FISH in paraffin-embedded diagnostic cores from eight patients analyzed by CGH. Furthermore, an independent series of 60 selected paraffin-embedded biopsy samples from prostate cancer patients diagnosed at our institution from June 1997 to June 1998 was used to confirm the prognostic significance of the CGH findings. All retrospective sample blocks were reevaluated by the same expert pathologist to assess the presence and grade of each tumor, after which all cases with Gleason score 7 (n = 24) were processed for interphase FISH analysis.

Treatment and follow-up data were obtained from the medical records. This study was approved by the institutional review board.

Comparative genomic hybridization. CGH analysis followed the procedure of Kallioniemi et al. (35), with modifications described previously (33, 36). Briefly, test (biopsy samples) and reference (peripheral blood lymphocytes from a male donor) DNA was extracted using standard methods and labeled in nick translation reactions using SpectrumGreen- and SpectrumRed-conjugated nucleotides (Vysis, Downers Grove, IL), after which probe lengths between 300 and 2,000 bp were obtained. Labeled tumor and reference DNA (1 μg each) were mixed with 30 μg unlabeled Cot-1 DNA (Life Technologies, Rockville, MD), ethanol precipitated, dried, and dissolved in hybridization buffer (Vysis). The probe mixture was denatured and hybridized to commercially available, normal metaphase slides (Vysis) for 2 to 3 days at 37°C in a moist chamber. After washing off excess probe, samples were counterstained with 4′,6-diamidino-2-phenylindole in an antifade solution (Vector Laboratories, Burlingame, CA). Single-color images corresponding to 4′,6-diamidino-2-phenylindole, green, and red fluorochrome hybridization signals were sequentially captured with a Cohu 4900 CCD camera using an automated filter wheel coupled to a Zeiss Axioplan fluorescence microscope (Zeiss, Oberkochen, Germany) and a CytoVision system version 2.7 (Applied Imaging, Santa Clara, CA). Ten high-quality metaphase spreads were selected for analysis in each case. Chromosomes were identified based on their inverted 4′,6-diamidino-2-phenylindole appearance and the relative signal intensity was determined along each chromosome. Data from the 10 cells were combined to generate average ratio profiles with 99% confidence intervals for each sample. We have recently adopted the use of dynamic standard reference intervals (37) for the scoring of all our CGH experiments. Our current dynamic standard reference interval was generated based on data from 10 normal versus normal hybridizations (totaling 110 cells). This interval was automatically scaled onto each sample and aberrations were scored whenever the case profile and the standard reference profile at 99% confidence did

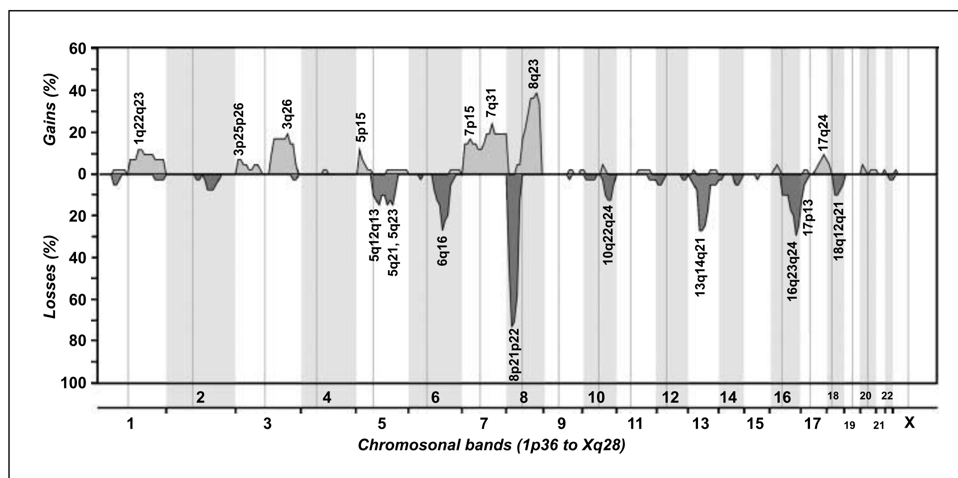

Fig. 1. Genomic findings in 61 prostate carcinomas detected in needle biopsies done in 100 prostate cancer suspects. Gains and losses of genetic material are depicted along all chromosomes (*X axis*), with the most frequently altered bands being indicated.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/12/13/3961/1963238/3961.pdf by guest on 09 December 2023

not overlap. For the scoring of amplifications, the threshold of 1.5 was chosen to account for the possible contamination with normal cells. Description of the CGH copy number changes followed the guidelines suggested in the International System for Human Cytogenetic Nomenclature (38).

Fluorescent* in situ *hybridization. Four-micron-thick sections were cut from a representative paraffin-embedded block off each patient onto SuperFrost Plus adhesion slides (Menzel-Glaser, Braunschweig, Germany). Sample processing, hybridization, and analysis were done according to standard protocols. Briefly, slides were deparaffinized in two series of xylol followed by two series of ethanol (5 minutes each), rinsed in 2× SSC, and placed in a solution of 1 mol/L sodium sulfocyanate at 80°C for 10 minutes (Merck, Darmstadt, Germany). The tissue was then digested with 6 mg/mL pepsin (Sigma-Aldrich, Steinheim, Germany) for 22 minutes at 37°C, after which slides were rinsed in 2× SSC and dehydrated in a series of ethanol. A dual-color probe flanking the *MYC* gene at 8q24 labeled with SpectrumGreen and SpectrumOrange and a centromeric probe for chromosome 18 labeled with SpectrumAqua (Vysis) were used for each sample. Slides were then placed in a Hybrite denaturation/hybridization system (Vysis) and codenatured at 80°C for 7 minutes. Hybridization took place for 18 hours at 37°C followed by posthybridization washes in 2× SSC/0.5% Igepal (Sigma-Aldrich) at 73°C for 5 minutes and 2× SSC/0.1% Igepal at room temperature for 3 minutes. Slides were counterstained with 4′,6-diamidino-2-phenylindole. Fluorescent images corresponding to 4′,6-diamidino-2-phenylindole, SpectrumGreen, SpectrumOrange, and SpectrumAqua were sequentially captured using the same equipment described for CGH analysis. Only intact, nonoverlapping nuclei were scored. An abnormal population was considered representative when at least three nuclei within the same microscope field presented a given aberration and at least 25 nuclei presented that particular aberration in the whole sample. For the purposes on this study, the final ratio between *MYC* and chromosome 18 centromere signals (*MYC*/CEP18 ratio) was computed for each sample (whenever several representative populations existed for a given tumor, the highest ratio was used).

Statistical analysis. For statistical purposes, prostate cancer samples were divided into three grade categories (Gleason scores ≤6, 7, and ≥8). Variables, such as age, presence or absence of genomic imbalances, and frequency of genomic changes detected in >10% of the cases, were tested for associations with histopathologic data. The χ^2 test, χ^2 test for trend, and Fisher exact test were applied according to the categorization of the variables. Kruskal-Wallis nonparametric test was used to assess the relationship of PSA levels, percentage of positive biopsy cores, and total number of genomic imbalances with tumor grade and clinical stage. A multivariate logistic regression (forward conditional setting) was done to evaluate the relative contribution of genetic and clinical variables to the prediction of follow-up status. Chromosomal aberrations seen in >10% of the cases, presence or absence of genomic imbalances, degree of genetic complexity, Gleason score, and clinical stage were entered in this model. Variables found to contribute significantly to the correct assessment of follow-up status were further tested for prognostic significance by constructing survival curves using the Kaplan-Meier method with log-rank test. $P < 0.05$ (two-sided) was considered to indicate statistical significance. All analyses were done using SPSS version 11.0 (SPSS, Chicago, IL). Unsupervised hierarchical clustering of the biopsy pairs based on the pattern of genomic alterations was done in J-Express Pro 2.5 (39) using average-linkage method with Pearson's correlation similarity measure.

Results

Histopathologic data. Carcinoma was detected on routine biopsy cores in 72 of the 100 individuals studied (Gleason score ≤6, 26 patients; Gleason score 7, 34 patients; Gleason score ≥8, 12 patients). Sixty-one of the 72 patients diagnosed with cancer displayed morphologic evidence of carcinoma in the research cores. The median diagnostic PSA (ng/mL) in samples with no evidence of tumor was 9.27 (range, 5.5-29.4) and increased across the three Gleason score categories [14.55 (range, 4.5-287.0), 29.45 (range, 5.3-158.0), and 63.25 (range, 8.2-570.0), respectively; $P < 0.001$]. There was a significant increase in the percentage of affected cores across the different Gleason categories (50%, 83%, and 100%; $P < 0.001$). Gleason grade was significantly associated with diagnostic clinical stage ($P = 0.002$). There were no significant differences between the clinical characteristics of the prospective and retrospective series of biopsies assessed in this study (i.e., in terms of age, PSA

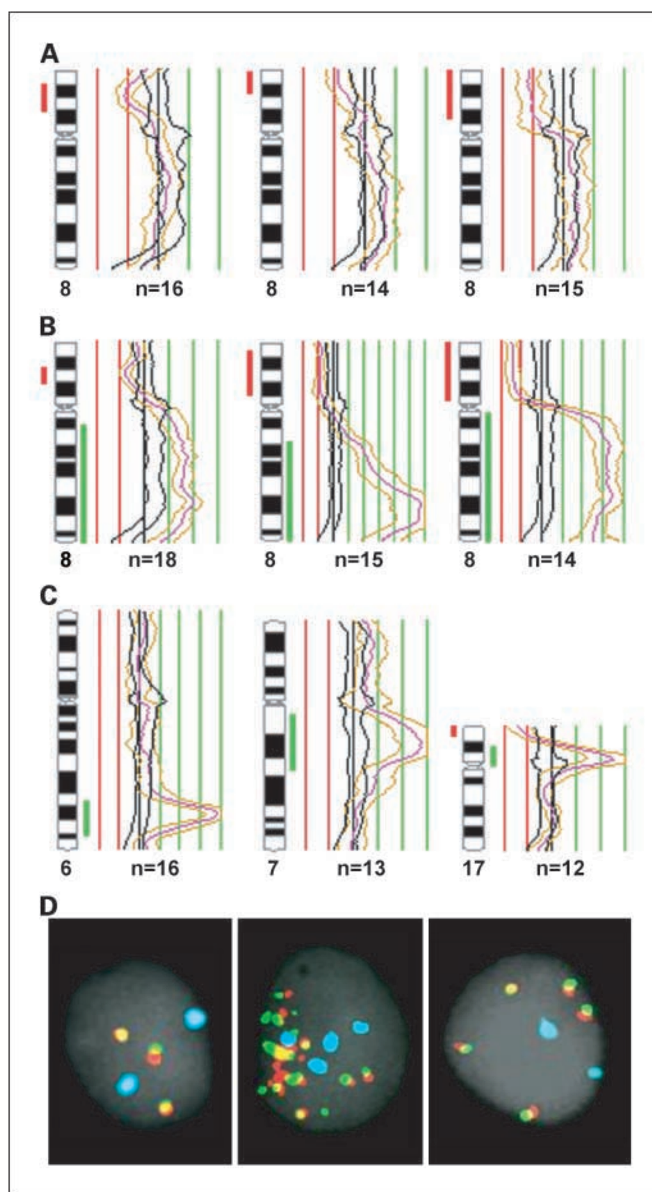

Fig. 2. Selected CGH and FISH findings on the prospective series of prostate cancer biopsies. *A,* different sizes of DNA copy number losses at chromosomal arm 8p with no 8q gain. *B,* different levels of DNA copy number gains in chromosome arm 8q with concomitant 8p loss. *C,* novel amplicons at chromosome arms 6q, 7q, and 17p. *D,* representative FISH images from the three biopsy samples displayed in (*B*), validating the CGH findings (3× *MYC*/2× CEP18, >10× *MYC*/4× CEP18, and 5× *MYC*/2× CEP18, respectively).

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/12/13/3961/1963238/3961.pdf by guest on 09 December 2023

levels, and frequencies of Gleason score and clinical stage categories).

Genetic findings. Among the 61 research cores with morphologic evidence of carcinoma, DNA copy number changes were detected in 41 (67%) samples (Table 1; Fig. 1). Overall, losses were seen in 38 (62%) tumors, whereas gains were seen in 27 (44%) cases and amplifications in 9 (16%) cases. Among the abnormal cases, the average number of genomic imbalances was 4.6, with losses (2.7) being more common than gains (1.9). Recurrent copy number losses were found at 8p (73% of the abnormal cases; Fig. 2A), 13q (32%), 6q (27%), 16q (27%), 5q (24%), 10q (15%), 17q (12%), and 18q (10%), whereas recurrent copy number gains were seen at 8q (39%; Fig. 2B), 7q (24%), 3q (22%), 7p (15%), 1q (15%), and 5p (12%). Amplifications were detected at 8q (6 cases; minimal region of overlap was 8q22q23), 8p11p12 (2 cases), and Xp22, 3q26, 4q21q22, 6q23q25, 7q11q22, 10q21, and 17p11p12 (1 case each; Fig. 2B and C). No DNA copy number aberrations were found in the 39 research cores without morphologic evidence of carcinoma. Genomic data from the 25 paired biopsy samples analyzed to assess tumor heterogeneity are shown in Table 1. For the 12 pairs of samples with comparable amounts of tumor content, the results show that all pairs with genomic changes shared at least one alteration, but several nonshared aberrations were also found. Overall, from a total of 69 alterations detected in these 12 paired cores, 35 (51%) were shared and 34 (49%) were not (an average of 3 shared and 3 nonshared changes per case). Unsupervised hierarchical clustering of the CGH data was able to correctly pair all but one of the informative pairs of samples (data not shown).

Genetically abnormal cell populations were detected in 20 of the 24 (83%) Gleason score 7 biopsies analyzed by FISH. A *MYC*/CEP18 ratio ≥1.5 was detected in 16 (67%) cases. FISH analyses of all 8 samples selected from the prospective series for validation of CGH findings showed concordant results for chromosomes 8 and 18 (Fig. 2B and D).

Correlations with clinical stage and tumor grade. A significant increase in the frequency of genomic aberrations was detected from well to poorly differentiated carcinomas (P = 0.02; Table 2). The increase in the number of losses was the main contributor to this association (P = 0.0004), but the number of gains also increased throughout the Gleason categories (P = 0.038). Amplifications were detected solely on samples with individual Gleason pattern 4 or 5 (P = 0.034) and the number of copy number losses was also associated with these histologic patterns (P = 0.018). The proportion of cases with DNA copy number changes, as well as the total number of aberrations, was significantly higher in samples with advanced clinical stage (P = 0.046 and 0.017, respectively). None of the specific genomic imbalances present in >10% of the cases was significantly correlated with tumor grade or clinical stage.

Survival data. Follow-up data of our prospective series are limited to an average of 35 months per patient (range, 22-52 months). Nine of the 72 patients with cancer have died and 3 others displayed clinical recurrences during this period. Binomial logistic regression showed that gain at 8q was the best predictor of poor prognosis (P < 0.001), as patients whose tumors displayed this alteration were 9.4 times more likely to have an adverse outcome (50% prediction accuracy regarding deceased patients, 75% overall). Genetic complexity, defined as tumors with more than two genomic imbalances, was also able to predict 62% of the deceased cases when tested alone (65% overall accuracy; P = 0.008), as patients with genetically complex tumors were four times more likely to have died from the disease than those with genetically less complex tumors. Survival analysis using the genetic variables highlighted by the regression model further showed that patients whose tumors displayed gain at 8q presented a significantly worse prognosis (P = 0.008; Fig. 3A) even when tumor grade and clinical stage were taken into account. In fact, when we considered only cases with Gleason score 7, the most troublesome category in terms of prognostic information, 8q gains were still significantly associated with poor survival (P = 0.011; Fig. 3B).

The independent prognostic value of 8q gain was confirmed by the FISH findings in the retrospective series of biopsy cores from patients with Gleason score 7, as those whose tumors displayed a *MYC*/CEP18 ratio ≥1.5 presented a significantly worse prostate cancer survival (P = 0.023; Fig. 3C). Interestingly,

Table 2. Genomic data and clinicopathologic associations in the 61 research biopsies with evidence of carcinoma collected from 100 prostate cancer suspects

	Gleason score				Clinical stage			
	4-6 (n = 18)	7 (n = 32)	8-10 (n = 11)	P	II (n = 35)	III (n = 12)	IV (n = 14)	P
Cases with copy number changes	39%	84%	63%	0.061	54%	92%	79%	0.046
Cases with gains	22%	59%	36%	0.244	34%	50%	64%	0.053
Cases with losses	27%	81%	63%	0.014	51%	92%	64%	0.196
Cases with amplifications	0%	19%	27%	0.034	11%	25%	14%	0.635
No. copy number changes*	1.1	4.3	2.7	0.020	2.0	5.0	4.0	0.017
No. gains*	0.5	1.7	1.4	0.038	0.8	1.6	2.1	0.063
No. losses*	0.6	2.6	2.3	<0.001	1.2	3.4	1.9	0.006
Cases with 8q gain	6%	34%	27%	0.107	23%	17%	38%	0.441
Genetically complex cases †	11%	53%	36%	0.074	26%	58%	50%	0.061

*Mean for each category is displayed.
†Tumors displaying three or more DNA copy number changes.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/12/13/3961/1963238/3961.pdf by guest on 09 December 2023

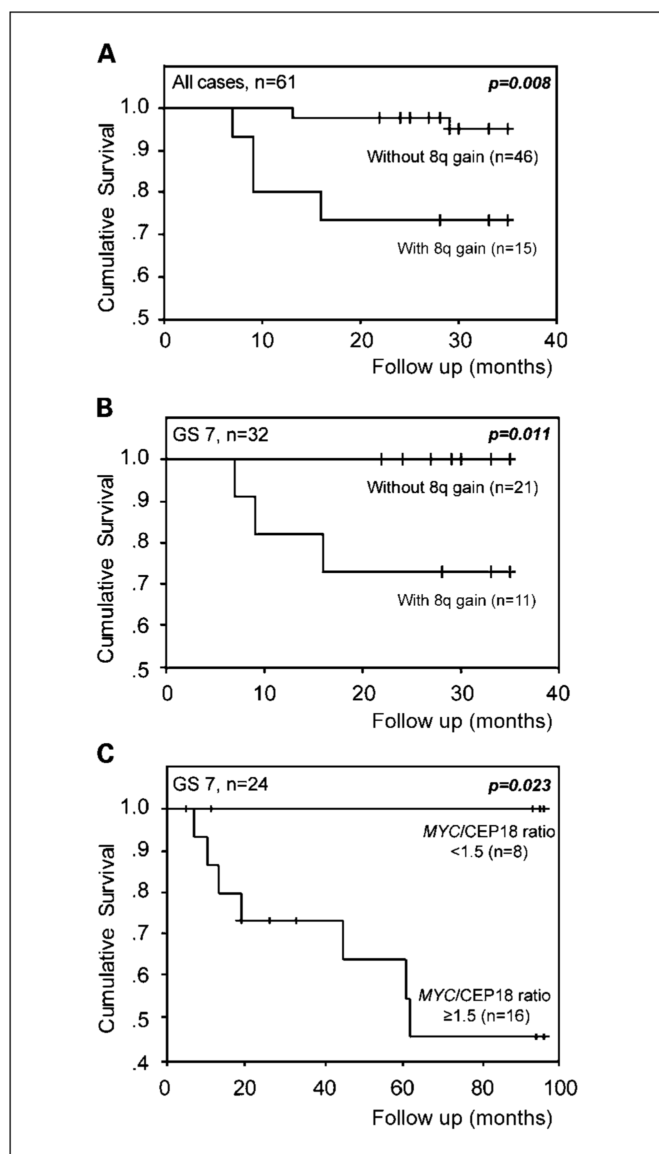

Fig. 3. Kaplan-Meier survival curves with log-rank tests according to the presence or absence of 8q gain and *MYC*/CEP18 ratio. *A,* survival according to 8q status by CGH on all prospective biopsies. *B,* survival according to 8q status by CGH on patients with Gleason score 7 from the prospective biopsy series. *C,* survival according to *MYC*/CEP18 ratios obtained using FISH on the retrospective biopsy series with Gleason score 7.

neither Gleason score nor clinical stage was associated with patient outcome after 35 months of follow-up (in both prospective and retrospective series), although both these clinical variables carried the expected prognostic significance after a follow-up time of 96 months in the retrospective series.

Discussion

We have recently shown that it is possible to consistently obtain whole genome information from prostate cancer sampled with ultrasound-guided sextant needle biopsies and that the genetic profile thus generated is consistent with the data available in the literature on prostatectomy samples (33). To evaluate the possible clinical application of this approach, we have studied a larger series of prospectively collected biopsies that better represent the population of prostate cancer suspects (individuals with increased PSA levels) presenting with and without malignant disease. Histopathologic evaluation revealed evidence of prostate carcinoma in a few more cases in the diagnostic than in the research biopsies (72 versus 61 of 100). This is best explained by the fact that six diagnostic versus two research biopsies were analyzed, and there is always a chance that these minute needle cores may miss small tumors. Indeed, all 11 patients without carcinoma in the research cores presented a low-grade cancer detected in <33% of the corresponding diagnostic cores. The representativeness of the individual biopsy cores is also strongly debated today, as tumor grade can be underestimated or overestimated depending on the fragment assessed and the experience of the pathologist (3 – 12). Many supposedly low-grade cancers actually display aggressive features when the prostatectomy specimen is assessed, but as most of these tumors are selected for surgical removal the effect on the patient may be reduced. On the other hand, up to 20% of the carcinomas are actually overgraded on biopsy and it could well be that at least some of these patients would benefit more from a "watchful waiting" approach than from an aggressive surgical treatment with known adverse side effects (40). Even with the aforementioned limitations in the histopathologic analysis of these minute samples, ultrasound-guided sextant biopsy remains the standard procedure to diagnose and grade prostate cancer. A more objective biomarker that could enhance its performance could markedly improve the clinical management of this disease.

Using CGH, we detected DNA copy number changes in more than two thirds of the biopsy cores with morphologic evidence of carcinoma and none in the biopsies without cancer. The overall profile of genomic alterations does not significantly differ from the literature data on prostate cancer with regard to the type of alterations (36). Nevertheless, we detected a higher frequency of gains compared with that found in organ-confined prostate cancers, which are normally better differentiated and genetically less complex. We found four novel prostate cancer amplicons at 6q23q25 and 7q11q22 (same patient), 10q21, and 17p11p12 as well as the previously reported amplified regions 3q26, 8p11p12, 8q, and Xp22. It is likely that genomic analysis of prostate cancer sampled by consecutive sextant biopsies encompasses a wider biological spectrum than those studies done on prostatectomy specimens alone, which is reflected in the fact that only 12 of 72 patients in our series were eligible for surgical resection of the prostate.

To evaluate the existence of tumor heterogeneity, one additional biopsy was analyzed in 25 of the prostate cancer patients. Only half of these displayed a percentage of tumor content in both biopsies that would allow the findings to be reliably compared. On average, each of these pairs shared three alterations, indicating a common clonal origin of the two samples. Additionally, unsupervised hierarchical clustering based on DNA copy number changes showed that a clonal relationship between the paired tumor samples could be shown in all but one patient. These 12 patients had large, poorly differentiated tumors detected in all diagnostic biopsy cores and most of the nonshared genetic alterations were infrequent in the whole series. These secondary aberrations likely arose by divergent clonal evolution, later during disease progression, and probably do not harbor clinically relevant information.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/12/13/3961/1963238/3961.pdf by guest on 09 December 2023

Our findings show that the total number of aberrations was significantly associated with increasing Gleason score and clinical stage. The previously mentioned difficulties in correctly grading prostate biopsies may account for the observed lack of association between specific genetic changes and tumor grade or clinical stage, as several genomic imbalances have been significantly associated with Gleason score in prostatectomy specimens (36). However, regression analysis showed that patients whose tumors displayed 8q gains or had more than two genetic copy number changes were more likely to have a poor outcome.

The survival data in our prospective study further strengthen the poor prognostic significance of 8q gains suggested in previous studies using FISH (23, 41) and CGH (13, 20, 42, 43), although the latter were retrospective and used mostly biochemical progression as the clinical end-point. Even when patients were stratified according to tumor grade or clinical stage, this genetic variable was able to identify patients with a worse outcome (Fig. 3), particularly within the group of tumors with Gleason score 7.

We confirmed the prognostic significance of 8q gain by FISH analysis in an independent, retrospective series of paraffin-embedded biopsies with much longer follow-up. The dual-color 8q probe we have used flanks the *MYC* gene (8q24.1) and targets two different regions of 260 and 400 kb separated by 1.72 Mb. This probe is expected to identify most prostate carcinomas with 8q gains and the dual-color labeling facilitates the scoring of copy number changes in archival specimens. To control for the ploidy of each case, we chose a chromosome 18 probe because the centromeric region of this chromosome is rarely affected in prostate cancer as opposed to other commercially available SpectrumAqua probes (chromosomes 8, 10, and 17). Besides confirming the CGH findings in the eight biopsies selected for that purpose, this three-color FISH assay showed in an independent series of Gleason score 7 needle biopsies that patients with tumor populations displaying *MYC*/CEP18 ratios ≥1.5 presented a significantly worse survival. Gleason score alone did not correlate with survival data on both the prospective and retrospective series of patients after 3 years of follow-up, something that is in accordance with literature data showing that the prognostic significance of this clinical variable is only evident after 5 years of follow-up time (44). It is therefore remarkable that 8q gain detected by either CGH or FISH is already significantly associated with death from disease after an average 35 months of follow-up, being particularly relevant for the large group of clinically localized prostate carcinomas with Gleason score 7, whose clinical behavior has been difficult to predict (45).

In summary, we show that relevant whole genome information can be obtained from prostate needle biopsies collected from prostate cancer suspects before any therapeutic action is taken. Whereas genetic complexity of cancer cells was significantly correlated with increasing tumor grade, survival analysis showed that 8q gain was the best indicator of poor prognosis even when Gleason score and clinical stage were taken into account. The use of tumor genetic information as an ancillary tool to histopathologic analysis of sextant biopsies may thus improve the clinical management of prostate cancer patients.

References

1. Terris MK, McNeal JE, Stamey TA. Detection of clinically significant prostate cancer by transrectal ultrasound-guided systematic biopsies. J Urol 1992;148:829–32.

2. Stamey TA. Making the most out of six systematic sextant biopsies. Urology 1995;45:2–12.

3. King CR, Long JP. Prostate biopsy grading errors: a sampling problem? Int J Cancer 2000;90:326–30.

4. Mills SE, Fowler JE, Jr. Gleason histologic grading of prostatic carcinoma. Correlations between biopsy and prostatectomy specimens. Cancer 1986;57:346–9.

5. Bostwick DG. Gleason grading of prostatic needle biopsies. Correlation with grade in 316 matched prostatectomies. Am J Surg Pathol 1994;18:796–803.

6. Peller PA, Young DC, Marmaduke DP, Marsh WL, Badalament RA. Sextant prostate biopsies. A histopathologic correlation with radical prostatectomy specimens. Cancer 1995;75:530–8.

7. Steinberg DM, Sauvageot J, Piantadosi S, Epstein JI. Correlation of prostate needle biopsy and radical prostatectomy Gleason grade in academic and community settings. Am J Surg Pathol 1997;21:566–76.

8. King CR. Patterns of prostate cancer biopsy grading: trends and clinical implications. Int J Cancer 2000;90:305–11.

9. Koksal IT, Ozcan F, Kadioglu TC, Esen T, Kilicaslan I, Tunc M. Discrepancy between Gleason scores of biopsy and radical prostatectomy specimens. Eur Urol 2000;37:670–4.

10. Noguchi M, Stamey TA, McNeal JE, Yemoto CM. Relationship between systematic biopsies and histological features of 222 radical prostatectomy specimens: lack of prediction of tumor significance for men with nonpalpable prostate cancer. J Urol 2001;166:104–9.

11. Smith EB, Frierson HF, Jr., Mills SE, Boyd JC, Theodorescu D. Gleason scores of prostate biopsy and radical prostatectomy specimens over the past 10 years: is there evidence for systematic upgrading? Cancer 2002;94:2282–7.

12. Sved PD, Gomez P, Manoharan M, Kim SS, Soloway MS. Limitations of biopsy Gleason grade: implications for counseling patients with biopsy Gleason score 6 prostate cancer. J Urol 2004;172:98–102.

13. Fu W, Bubendorf L, Willi N, et al. Genetic changes in clinically organ-confined prostate cancer by comparative genomic hybridization. Urology 2000;56:880–5.

14. Alers JC, Krijtenburg PJ, Vis AN, et al. Molecular cytogenetic analysis of prostatic adenocarcinomas from screening studies: early cancers may contain aggressive genetic features. Am J Pathol 2001;158:399–406.

15. Wolter H, Gottfried HW, Mattfeldt T. Genetic changes in stage pT_2N_0 prostate cancer studied by comparative genomic hybridization. BJU Int 2002;89:310–6.

16. Chu LW, Troncoso P, Johnston DA, Liang JC. Genetic markers useful for distinguishing between organ-confined and locally advanced prostate cancer. Genes Chromosomes Cancer 2003;36:303–12.

17. Visakorpi T, Kallioniemi AH, Syvänen AC, et al. Genetic changes in primary and recurrent prostate cancer by comparative genomic hybridization. Cancer Res 1995;55:342–7.

18. Cher ML, Bova GS, Moore DH, et al. Genetic alterations in untreated metastases and androgen-independent prostate cancer detected by comparative genomic hybridization and allelotyping. Cancer Res 1996;56:3091–102.

19. Nupponen NN, Kakkola L, Koivisto P, Visakorpi T. Genetic alterations in hormone-refractory recurrent prostate carcinomas. Am J Pathol 1998;153:141–8.

20. Alers JC, Rochat J, Krijtenburg PJ, et al. Identification of genetic markers for prostatic cancer progression. Lab Invest 2000;80:931–42.

21. Ross JS, Sheehan CE, Ambros RA, et al. Needle biopsy DNA ploidy status predicts grade shifting in prostate cancer. Am J Surg Pathol 1999;23:296–301.

22. Sebo TJ, Cheville JC, Riehle DL, et al. Predicting prostate carcinoma volume and stage at radical prostatectomy by assessing needle biopsy specimens for percent surface area and cores positive for carcinoma, perineural invasion, Gleason score, DNA ploidy and proliferation, and preoperative serum prostate specific antigen: a report of 454 cases. Cancer 2001;91:2196–204.

23. Takahashi S, Qian J, Brown JA, et al. Potential markers of prostate cancer aggressiveness detected by fluorescence *in situ* hybridization in needle biopsies. Cancer Res 1994;54:3574–9.

24. Henke RP, Krüger E, Ayhan N, Hübner D, Hammerer P. Frequency and distribution of numerical chromosomal aberrations in prostatic cancer. Hum Pathol 1994;25:476–84.

25. Henke RP, Hammerer P, Graefen M, et al. Interphase cytogenetic study of preoperative core biopsies for the prediction of early serum prostate specific antigen recurrence after radical prostatectomy of clinically localized prostate carcinoma. Cancer 1998;83:977–88.

26. Al Maghrabi J, Vorobyova L, Toi A, Chapman W, Zielenska M, Squire JA. Identification of numerical chromosomal changes detected by interphase fluorescence *in situ* hybridization in high-grade prostate intraepithelial neoplasia as a predictor of carcinoma. Arch Pathol Lab Med 2002;126:165–9.

27. Matsuyama H, Pan Y, Skoog L, et al. Deletion mapping of chromosome 8p in prostate cancer by fluorescence *in situ* hybridization. Oncogene 1994;9:3071–6.

28. Bastacky S, Cieply K, Sherer C, Dhir R, Epstein JI. Use of interphase fluorescence *in situ* hybridization in prostate needle biopsy specimens with isolated high-grade prostatic intraepithelial neoplasia as a predictor of prostate adenocarcinoma on follow-up biopsy. Hum Pathol 2004;35:281–9.

29. Jeronimo C, Usadel H, Henrique R, et al. Quantitation of GSTP1 methylation in non-neoplastic prostatic

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/12/13/3961/1963238/3961.pdf by guest on 09 December 2023

tissue and organ-confined prostate adenocarcinoma. J Natl Cancer Inst 2001;93:1747–52.

30. Harden SV, Sanderson H, Goodman SN, et al. Quantitative GSTP1 methylation and the detection of prostate adenocarcinoma in sextant biopsies. J Natl Cancer Inst 2003;95:1634–7.

31. Dreher T, Zentgraf H, Abel U, et al. Reduction of PTEN and $p27^{kip1}$ expression correlates with tumor grade in prostate cancer. Analysis in radical prostatectomy specimens and needle biopsies. Virchows Arch 2004;444:509–17.

32. Zhou M, Tokumaru Y, Sidransky D, Epstein JI. Quantitative GSTP1 methylation levels correlate with Gleason grade and tumor volume in prostate needle biopsies. J Urol 2004;171:2195–8.

33. Teixeira MR, Ribeiro FR, Eknaes M, et al. Genomic analysis of prostate carcinoma specimens obtained via ultrasound-guided needle biopsy may be of use in preoperative decision-making. Cancer 2004;101:1786–93.

34. Gleason DF, Mellinger GT. Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. J Urol 1974;111:58–64.

35. Kallioniemi OP, Kallioniemi A, Piper J, et al. Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. Genes Chromosomes Cancer 1994;10:231–43.

36. Ribeiro FR, Diep CB, Jeronimo C, et al. Statistical dissection of genetic pathways involved in prostate carcinogenesis. Genes Chromosomes Cancer 2006;45:154–63.

37. Kirchhoff M, Gerdes T, Rose H, Maahr J, Ottesen AM, Lundsteen C. Detection of chromosomal gains and losses in comparative genomic hybridization analysis based on standard reference intervals. Cytometry 1998;31:163–73.

38. ISCN 1995. An international system for human cytogenetic nomenclature. Basel: S. Karger; 1995.

39. Dysvik B, Jonassen I. J-Express: exploring gene expression data using Java. Bioinformatics 2001;17:369–70.

40. Steineck G, Helgesen F, Adolfsson J, et al. Quality of life after radical prostatectomy or watchful waiting. N Engl J Med 2002;347:790–6.

41. Sato K, Qian J, Slezak JM, et al. Clinical significance of alterations of chromosome 8 in high-grade, advanced, nonmetastatic prostate carcinoma. J Natl Cancer Inst 1999;91:1574–80.

42. Steiner T, Junker K, Burkhardt F, Braunsdorf A, Janitzky V, Schubert J. Gain in chromosome 8q correlates with early progression in hormonal treated prostate cancer. Eur Urol 2002;41:167–71.

43. Van Dekken H, Alers JC, Damen IA, et al. Genetic evaluation of localized prostate cancer in a cohort of forty patients: gain of distal 8q discriminates between progressors and nonprogressors. Lab Invest 2003;83:789–96.

44. Humphrey PA. Gleason grading and prognostic factors in carcinoma of the prostate. Mod Pathol 2004;16:292–306.

45. DeMarzo AM, Nelson WG, Isaacs WB, Epstein JI. Pathological and molecular aspects of prostate cancer. Lancet 2003;361:955–64.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/12/13/3961/1963238/3961.pdf by guest on 09 December 2023