European Urology

European Urology 41 (2002) 167–171

Gain in Chromosome 8q Correlates with Early Progression in Hormonal Treated Prostate Cancer

Thomas Steiner*, Kerstin Junker, Frank Burkhardt, Andreas Braunsdorf, Volker Janitzky, Joerg Schubert

Department of Urology, Friedrich-Schiller-University, D-07740 Jena, Germany

Accepted 12 September 2001

Abstract

Objective: Development and especially hormone refractant progression of prostate cancer are incompletely understood. Clinical studies evaluating genetic aberrations of prior therapy biopsies in correlation with progression data in patients receiving hormone therapy for prostate cancer have not been performed until now.

Methods: After DNA isolation from histological sections of primary prostate cancer biopsies, comparative genomic hybridization (CGH) was performed according to standard protocols. Primary staging, clinical course and PSA levels of the patients were assessed.

Results: CGH was performed on 28 primary prostate cancer samples. After a mean follow-up of 36 months 11 (39%) of the patients showed progression of disease under hormonal treatment. In patients without and with progression we found the following results, respectively: losses of 6q (41/36%), 8p (41/45%), 16q (23/18%), 18q (30/9%), and gains of 8q (12/64%; $P < 0.0001$) and 17 (47/26%).

Conclusions: Gain of 8q is found predominantly in primary core biopsies of local advanced or metastasized prostate cancers. It shows in univariate analysis significant correlation with progression in hormone treated prostate cancer. This fact suggests that gain in 8q represents a marker of aggressiveness in prostate cancer.

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Keywords: Prostate cancer; CGH; Hormone treatment; Prognosis

1. Introduction

Prostate cancer is the most common malignancy and the second leading cause of cancer death in men in western countries [1]. In patients not suitable for curative treatment options, hormone deprivation represents standard therapy. The molecular genetic events responsible for the initiation and progression of prostate cancer remain largely unknown [2]. Because prostate adenocarcinoma has extensive variability in clinical behavior, accurate prediction of the probability of tumor progression and of patient survival is the major goal of current prostate cancer research [3].

Conventional cytogenetic studies failed to demonstrate typical genetic aberrations in prostate cancer because of suboptimal in vitro growth of tumor cells. Another drawback of these techniques was the fact that proliferating cells in culture are not necessarily representative for the predominant population in the primary tumor [4].

During the past decade, new molecular genetic techniques such as fluorescence in situ hybridization (FISH) and more recently comparative genomic hybridization (CGH) have resulted in numerous reports regarding specific chromosomal aberrations in prostate tumors [5]. CGH, first described by Kallioniemi in 1992, is a technique with several potential advantages over conventional techniques [6]. It is similar to conventional cytogenetics where the entire genome of individual tumors is screened for changes; however, it does not require cell culture—the starting material is

* Corresponding author. Tel. +49-3641-935004; Fax: +49-3641-935003.
E-mail address: thomas.steiner@med.uni-jena.de (T. Steiner).

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PII: S0302-2838(01)00030-6

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isolated directly from tumor tissue. CGH can distinguish between gains and losses [7]. The possibility of using paraffin embedded tumor tissue represents another advantage of CGH [8].

Until now published data on CGH analysis in prostate cancer focused either on aberrations in primary tumors in comparison to tumors progressing under hormonal treatment [9] or genetic changes in primary tumors in correlation to risk of progression after radical prostatectomy [10].

The aim of this study was to define genetic alterations in primary prostate cancer biopsies in correlation to clinical course of disease in hormonal treated patients.

2. Materials and methods

2.1. Patients

A total of 28 patients with histologically confirmed prostate cancer in 1993/94 were enrolled in this study. All patients were not suitable for radical treatment of prostate cancer because of tumor staging, age or concomitant diseases. PSA levels at time of diagnosis, grading and primary clinical stage were assessed. All patients underwent hormonal treatment (LHRH analogs +/− anti-androgens; orchiectomy). Patients were followed for clinical and PSA course, median follow-up was 36 months. Besides development of new metastasis, raising PSA values under therapy were estimated as progression of disease.

2.2. DNA extraction

Tumor cells were dissected from paraffin sections of core biopsies after examination by a pathologist. DNA was extracted using a commercial kit (Qiagen). Normal DNA was isolated from blood cells collected from control individuals.

2.3. Amplification and labeling of DNA

To obtain sufficient amounts of tumor DNA for CGH analysis, DNA was amplified according to a modified protocol for DOP-PCR [11,12] using Sequenase in the first 8 cycles of nonspecific PCR, followed by additional 30 cycles under specific conditions using TaqPolymerase (Stoffel fragment). It was shown in earlier studies that DOP-PCR give the same results by CGH as CGH without DOP-PCR (own unpublished data). Labeling of tumor DNA and normal DNA was performed in an additional 20 cycles using Biotin-16dUTP and Digoxigenin-11dUTP, respectively.

Table 1

Patient characteristics, prior therapy PSA levels, clinical course of disease, detected chromosomal aberrations in prior therapy biopsies (dim: loss of chromosome, enh: gain of chromosome, n.d.: not done)

Number	Age at the time of diagnosis (years)	Grading (core biopsy)	PSA (prior therapy) (ng/ml)	Mts (yes: M+; no: M0; unknown: Mx)	Chromosomal aberrations (core biopsy)	Progress (yes: +; no: −)
1	66	GIIb	779	M+	dim(6q,Xq), enh(8q,10q)	+
2	81	GII	449	M+	dim(1p,4p,5q,6q,7q,8p,12q,13,18q), enh(3q,4q,5p, 8q,11p,15p)	+
3	83	GIIb	19	Mx	enh(18q)	+
4	84	GIII	29	M0	dim(1q,6q;16q,18q), enh(16p,17,18)	−
5	64	GII	5	M0	dim(7q,18q), enh(17)	−
6	69	GIIa	16	M0	dim(6q,18q,20p), enh(17)	−
7	72	GIIb	52	M0	No aberration	−
8	82	GII	n.d.	Mx	dim(8p,16p,18q), enh(8q,16q)	+
9	64	GIII	865	M+	dim(2q,6q,8p,13q), enh(3q,7q,8q,11q,12q)	+
10	80	GII	34	M0	dim(8p), enh(9q)	−
11	72	GIII	419	M+	dim(2q,4p,6q,8p,9p,11p,13q), enh(7q,8q,11q,17)	+
12	80	GII	70	M0	dim(4q,6q,8p,9q,18q)	−
13	69	GIIa	7	M0	No aberration	−
14	71	GII	12	M0	dim(6q), enh(15,17)	−
15	77	GIIa	2	M0	dim(6q,7q)	−
16	87	GIII	100	Mx	dim(8p,9p,11p,14q,16q), enh(1p,3p,6p,8q,10q,13,17)	+
17	82	GIII	n.d.	M0	dim(8q,16q), enh(7)	−
18	73	GIIb	20	M0	dim(16q), enh(17)	+
19	71	GII	6	M+	dim(8p,16q), enh(17)	−
20	86	GIII	17	Mx	No aberration	+
21	79	GIIb	113	Mx	dim(6q,8p,9p,16,18q)	−
22	78	GII	30	M0	dim(8p,9p,18q), enh(8q)	−
23	78	GIIb	42	M0	enh(17)	−
24	79	GII	15	M0	enh(17)	−
25	71	GII	74	M+	dim(1q), enh(8q)	+
26	81	GIII	19	M0	dim(2q,13q), enh(11p)	+
27	72	GIIb	11	M0	dim(6p,8p,16p), enh(17)	−
28	83	GI	29	M0	dim(10q), enh(18p)	−

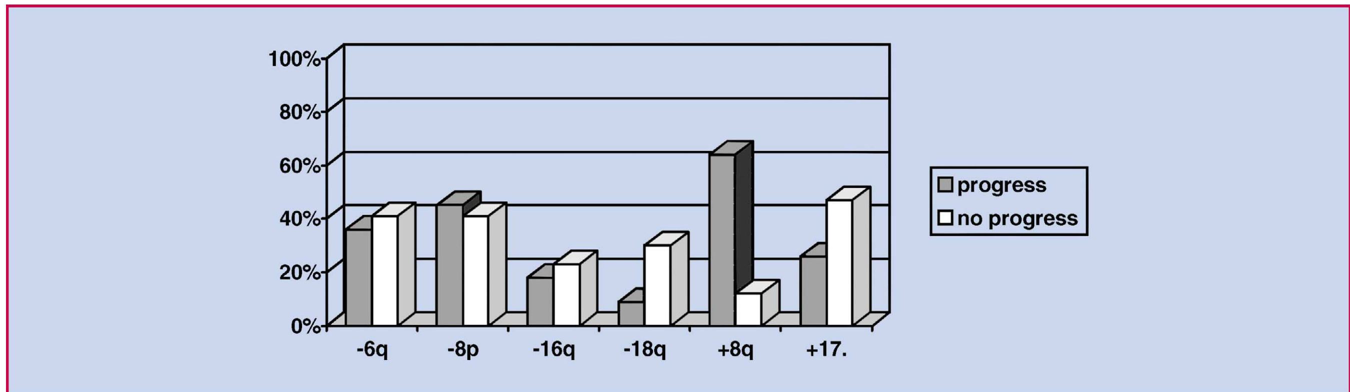

Fig. 1. Chromosomal aberrations in primary prostate biopsies; data are shown dependent on clinical course of disease.

2.4. CGH

1 µg of both tumor DNA and normal DNA was hybridized with 50 µg of Cot-1 DNA on normal metaphases at 37 °C for 48 h. Detection of fluorescent signals was carried out with avidin-FITC and anti-Digoxigenin-rhodamine. DAPI-antifade was used for counterstaining of chromosomes. Fifteen metaphases per case were analyzed using an Axioplan microscope (Zeiss, Germany) and the computer system from ''Metasystems'' (Germany).

2.5. Data analysis

Data analysis was performed with MS-DOS-based PCs using SPSS for Windows (Microsoft), Harvard Chart and Harvard Graphics (SPs).

Exact Fischer's test was used to evaluate differences of chromosomal alterations frequency in the groups with and without progression for each chromosome. The statistical significance of difference in number of aberrations per tumor in distinct tumor groups was tested by the *U*-test.

3. Results

Median age of patients at time of diagnosis was 76.3 years (range 64–87), median PSA level prior therapy 81.0 ng/ml (range 2–865). All patients showed primary response to therapy. Response was defined by decrease of PSA value to <4 ng/ml. After a mean follow-up of 36 months 11 (39%) of the patients had developed progression of disease under hormonal treatment. Age at time of diagnosis was similar in patients without and with progression (75.8 years (range 64–84) versus 76.9 years (range 64–87)). Mean PSA level prior therapy in patients without and with progression were 31.4 ng/ml (range 2–113) and 269.3 ng/ml (range 6–869), respectively ($P < 0.0001$). Table 1 gives an overview on PSA and progression data of the 28 patients.

The mean total number of changes per tumor in patients without progression was 3.8 and in patients with progressive disease 5.3. Overall, the most frequently gained chromosome arms were 8q (32%) and 17 (43%). The most frequent lost chromosome arms were 6q (40%), 8p (32%), 16q (21%) and 18q (21%). In patients without and with progression we found the following results, respectively: losses of 6q (41/36%), 8p (41/45%), 16q (23/18%), 18q (30/9%), and gains of 8q (12/64%; $P < 0.0001$) and 17 (47/26%). Fig. 1 summarizes the detected chromosomal aberrations, the CGH Profiles of patient no. 2 are shown in Fig. 2. Ratio losses/gains in primary prostate cancer biopsies from nonprogressive and progressive tumors was 2:1 and 1:1, respectively.

4. Discussion

The detected chromosomal aberrations in this study of primary prostate cancer biopsies are in good accordance with previously published FISH and CGH data on prostate cancer [13,14].

In 1998 Alers et al. showed by longitudinal evaluation of cytogenetic aberrations in prostate cancers using in situ hybridization (primary tumors; local recurrences after radical prostatectomy; distant metastases), that significant increase is seen for gain of chromosome 7 and/or 8 in the primary tumor tissue from the reference group (0%), to the recurrence group (27%), to the distant metastasis group (33%) [2]. In partial contradiction are results of a study published in 1998 too. The authors suggested that tumor suppressor genes relevant for prostate carcinogenesis might exist on 8p and allelic loss for the region at 8p22 is significantly associated with systemic cancer progression and cancer-specific survival after radical prostatectomy [3]. Indeed, further studies have confirmed this theory [10]. Taken together, the literature results suggest that inactivation of one or more tumor suppressor genes

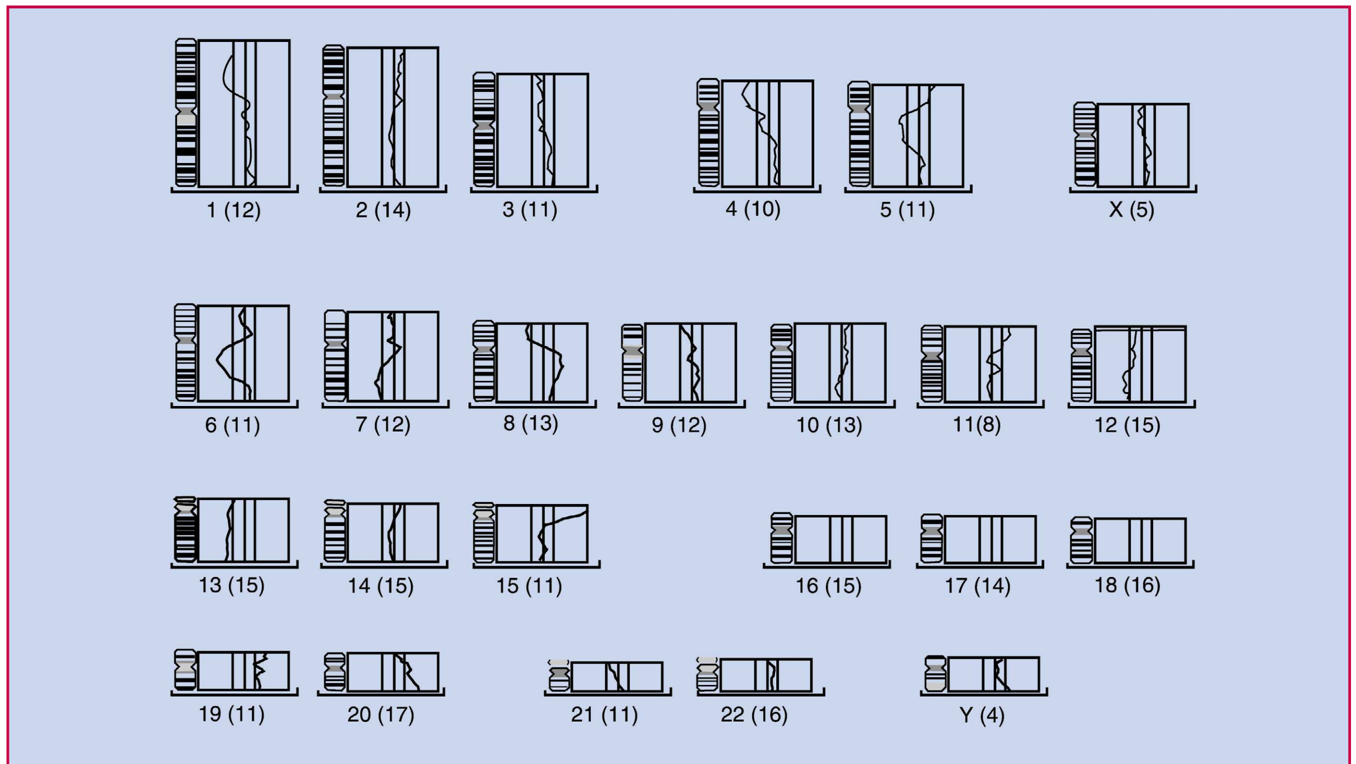

Fig. 2. CGH Profiles showing multiple gains and losses of chromosome parts in a tumor with progression (patient no. 2): profile at the middle line: no changes; shift to the right borderline: gain of chromosome or part of a chromosome; shift to the left borderline: loss of chromosome or part of a chromosome.

mapped to 8p may play a significant role in the initiation and progression of prostate cancer.

In this study, we evaluated cancer progression data in patients receiving hormone treatment for prostate cancer. Loss of 8p is detected in about 42% of prior therapy biopsies independent of cancer progression. This is in conformity with the suggestion that losses of tumor suppressor genes are early events in prostate cancer carcinogenesis.

Gain of 8q was described as a marker of prostate cancer progression already in 1995. In a study of 44 prostatectomy specimens in 3/4 primary tumors of patients with positive lymph nodes, CGH detected gain of 8q and in 1/40 without lymph node metastasis, respectively. Furthermore, gain of 8q was observed in 8/9 recurrent tumors after radical prostatectomy [15]. In 2000, gain of 7pq and/or 8q was identified by Alers et al. as a potential genetic discriminator between progressors and nonprogressors after radical surgery [16]. Nupponen et al. detected gain of 8q in 27 (72.5%) of 37 hormone refractory prostate carcinomas [17]. Alers et al published data in 1997 which suggest that gain of chromosome 8 is related to local tumor growth and overrepresentation of 8q sequences is involved in metastatic spread to the bone [18]. A recently published study compared CGH profiles of early, localized tumors with large adenocarcinomas of the prostate. Interestingly, chromosomal alterations which were found to be potential biomarkers for tumor aggressiveness in previous studies, i.e. gain of 7 and/or 8q, occurred already in the small and intermediate cancers. They concluded that early localized tumors, as detected by screening programs, harbor cancers with aggressive genetic characteristics [19].

While summarizing published data, it is obvious that aberrations of chromosome 8 are implicated in prostate cancer carcinogenesis and progression. In this study, we frequently found gain in 8q in primary prostate cancer core biopsies of patients with local advanced and/or metastasized tumors which progressed under hormone treatment.

Our data suggest that gain of 8q seems to be a late event in prostate cancer carcinogenesis. Detection of this aberration in primary prostate cancer core biopsies shows significant correlation with progression in hormone treated prostate cancer. These results support hypothesis that gain in 8q represents a marker of aggressiveness.

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