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# Genetic Alterations in Hormone-Refractory Recurrent Prostate Carcinomas

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**To study the genetic basis of tumor progression, we have screened 37 hormone-refractory prostate carcinomas for genetic changes by comparative genomic hybridization (CGH). All recurrent tumors showed genetic aberrations, with a mean total number of changes per tumor of 11.4 (range, 3 to 23). The most common genetic aberrations were losses of 8p (72.5%), 13q (50%), 1p (50%), 22 (45%), 19 (45%), 10q (42.5%), and 16q (42.5%) and gains of 8q (72.5%), 7q (40%), Xq (32.5%), and 18q (32.5%). The CGH results were further validated with fluorescence *in situ* hybridization (FISH) using probes for pericentromeric regions of chromosomes 7, 8, and 18 as well as probes for caveolin (7q31), c-myc (8q24), and bcl-2 (18q21.3). In addition, the samples had previously been analyzed for androgen receptor gene copy number. CGH and FISH results were concordant in 78% of cases. Seventeen of twenty-two tumors showed an increased copy number of c-myc by FISH. However, only 5 of 17 (29%) of the cases showed high-level (more than threefold) amplification. Both CGH and FISH findings suggested that in most of the cases 8q gain involves the whole q-arm of the chromosome. Four of seventeen (24%) cases showed increased copy number of bcl-2 by FISH; however, no high-level amplifications were found. To evaluate the clonal relationship of the primary and recurrent tumors, six primary-recurrent tumor pairs from the same patients were studied by CGH. In three of six cases (50%), the recurrent tumor had more than one-half of the aberrations found in the corresponding primary tumor, indicating a close clonal relationship. In the rest of the cases, such a linear clonal relationship was less evident. Altogether, these results suggest that recurrent prostate carcinomas are genetically unstable. The resulting heterogeneity may well underlie the poor responsiveness of hormone-refractory tumors to treatment.** ***(Am J Pathol 1998, 153:141–148)***

Prostate cancer is the most common malignancy among men in many Western industrialized countries. Despite the improved early diagnosis of prostate cancer, approximately one-third of the patients are still diagnosed at a clinically advanced stage.[1] For these patients, androgen withdrawal remains the only effective treatment. Most of the prostate cancer patients initially respond to hormonal therapy,[2] but eventually the disease will progress. And there are no effective second-line treatments for such hormone-refractory tumors.[2]

The mechanisms that lead to progression of prostate cancer during endocrine treatment are poorly understood. Several hypotheses on the molecular mechanisms of tumor recurrence have been suggested. These include overexpression of the bcl-2 oncogene,[3–5] activating mutations in the androgen receptor (AR) gene,[6,7] and amplification and overexpression of the AR gene.[8,9]

Comparative genomic hybridization (CGH) is a fairly new molecular cytogenetic method that allows detection of DNA sequence copy number changes throughout the genome in a single hybridization.[10–12] CGH is helpful in defining chromosomal regions that may harbor amplified oncogenes or deleted tumor suppressor genes (TSGs). Thus, CGH provides a starting point for positional cloning of cancer-related genes. Several common malignancies, including prostate cancer,[13–16] have already been studied by CGH. These studies have also led to the identification of actual amplified genes in cancer.[8,17–19] As CGH detects only clonal genetic aberrations, it is also useful in the investigation of the clonal evolution of tumor progression.[20]

To identify genetic aberrations that may underlie the progression of prostate cancer during endocrine therapy, we have now screened 37 hormone-refractory prostate tumors for genetic changes by CGH. In addition, we have studied the genetic relationship of six primary-recurrent tumor pairs. CGH findings were also further studied by fluorescence *in situ* hybridization (FISH) and locus-specific probes.

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## Materials and Methods

### Tumor Specimens

The material consisted of 37 formalin-fixed, paraffin-embedded recurrent prostate carcinomas obtained from the Tampere University Hospital. According to histological grade,[21] there were 1 grade I, 15 grade II, and 21 grade III recurrent tumors. According to the TNM stage distribution of the recurrent prostate carcinomas, there were 1 T1NXMO, T2NXMX, T2NXM1; 5 T3NXMX; 6 T3NXMO, T3NXM1; 7 T4NXMO; 3 T4NXMX; and 5 T4NXM1. All samples were transurethral resection specimens taken from patients who had received only endocrine therapy (orchiectomy (28 cases), estrogen (3 cases), luteinizing hormone-releasing hormone agonist (1 case), and combinations of these therapies (5 cases)) and who had experienced local progression as evidenced by new onset of urethral obstruction. The choice of endocrine therapy instead of surgical treatment had been based either on clinical stage of the disease or general condition of the patients. An average time from the diagnosis (beginning of hormonal therapy) to progression was 44 (range, 8 to 113) months and from the progression to death was 29 (1 to 101) months. In addition, six primary-recurrent tumor pairs (five paraffin embedded, one freshly frozen) were available. The primary tumors (transurethral resection specimens) were taken before any treatment, and the recurrent tumors were from the same patients at the time of local relapse (urethral obstruction).

Five-micron sections were cut from tumor blocks and stained with hematoxylin and eosin to detect the histological representativeness of the malignant tissue. High molecular weight tumor DNAs for CGH and interphase nuclei for FISH were isolated from paraffin-embedded tumor blocks as described before.[22,23]

### CGH

CGH was done as described before.[12,22] Briefly, DNA samples from prostate tumors were labeled with fluorescein isothiocyanate (FITC)-dUTP (DuPont, Boston, MA) and normal reference male DNA with Texas Red-dUTP (DuPont) using nick translation. Labeled DNAs (400 ng) were hybridized to normal male lymphocyte metaphase slides (Vysis, Downers Grove, IL) together with unlabeled Cot-1 DNA (10 $\mu$g; Gibco BRL, Gaithersburg, MD). After hybridization, the slides were washed and counterstained with an anti-fade solution containing 4,6-diamidino 2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA).

### Digital Image Analysis

Five high quality metaphases from each hybridization were captured using a Xillix CCD camera (Xillix Technologies Corp., Vancouver, British Columbia, Canada) mounted on an Olympus BX50 epifluorescence microscope (Tokyo, Japan) and interfaced to a Sun LX workstation (Sun Microsystems Computer Corp., Mountain View, CA). Relative DNA sequence copy number changes were detected by analyzing the fluorescence intensities of green (tumor) and red (normal) signals along the length of all chromosomes in the metaphase spreads using Quips CGH analysis program (Resource of Molecular Cytogenetics, Lawrence Berkeley National Laboratory, Berkeley, CA) based on the Scilimage program (TNO, Delft, The Netherlands). CGH results were plotted as a series of green-to-red ratio profiles and the interpretation of results followed previously described guidelines.[12] Hybridizations of FITC-labeled normal male DNA against Texas-Red-labeled normal female DNA, in each hybridization batch, were used as negative controls. The mean green-to-red ratio and corresponding SD for all autosomes remained between 0.85 and 1.15. Based on these control hybridizations, chromosomal regions with a mean ratio of 0.85 or less were considered lost, and those with a ratio 1.15 or more were considered gained in the prostate tumors. Chromosome Y was excluded from CGH analysis. The MCF-7 breast cancer cell line was used as a positive control in each hybridization batch.

### FISH

Interphase FISH was performed with locus-specific probes for caveolin (obtained by screening the human PAC library with PCR using primers specific to caveolin) located at 7q31, c-myc (P1, c-myc, RMC08P001, Lawrence Berkeley National Laboratory) located at 8q24, and bcl-2 (obtained by screening the human P1 library with PCR using primers specific to bcl-2) at 18q21.3. Chromosomes 7 (p7$\alpha$tet), 8 (pJM128), and 18 (p18r) pericentromeric alphoid probes were used as reference probes. Locus-specific probes were labeled with digoxigenin-11-dUTP (myc) (Gibco BRL) or biotin-14-dATP (caveolin, bcl-2; Gibco BRL) and centromeric probes with FITC-dUTP (chromosome 8; DuPont) or Texas-Red-dUTP (chromosomes 7 and 18; DuPont). FISH was performed as described in detail elsewhere.[23] Before FISH, the slides were pretreated by heating in 59% glycerol/0.1X standard saline citrate (SSC; pH 7.5) solution at 90°C for 3 minutes to improve hybridization efficiency. After hybridization, the slides were washed and counterstained with an anti-fade solution containing DAPI (Vector). In addition, 30 tumors had earlier been analyzed for chromosome X centromere and AR gene copy number by FISH.[9]

The entire slide was first scanned through, and 120 to 150 randomly chosen individual nuclei were scored in detail to calculate signals for the locus-specific probes and centromeric probes. Control hybridizations to normal lymphocyte metaphase preparations were done to ascertain that the probes recognized a single-copy target and to evaluate the hybridization efficiencies of the probes.

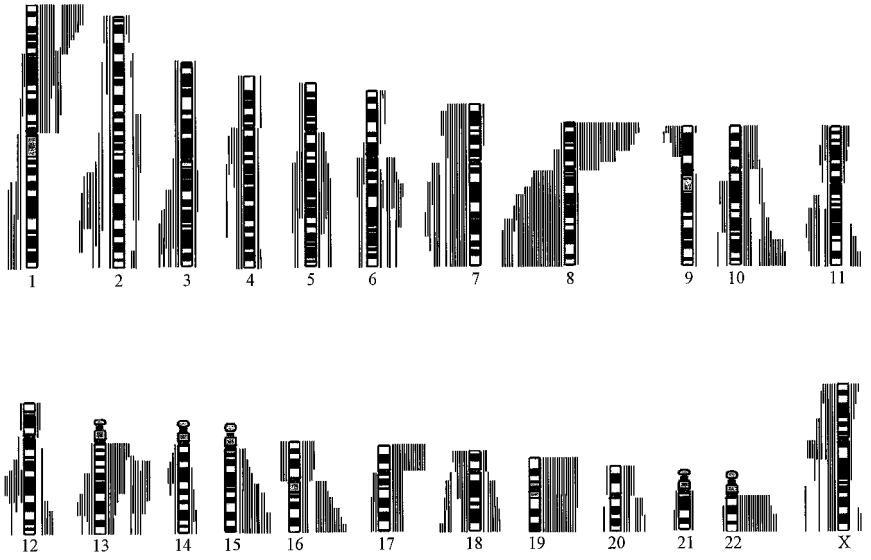


Figure 1. Summary of all DNA sequence copy number changes in 37 recurrent prostate carcinomas detected by CGH. Gains are shown on the left side of the chromosome ideograms and losses on the right. One bar represents one tumor. Chromosome Y was excluded from the analysis.

## Results

### Genetic Changes in Recurrent Prostate Carcinomas

The mean total number of changes per tumor in recurrent prostate cancer was 11.4 (range, 3 to 23). The average number of gains per tumor was 4.5 (range, 0 to 13) and of losses was 6.6 (range, 1 to 15).

The most frequently lost chromosome arms were (Figure 1) 8p (73%), 1p (54%), 13q (51%), 22q (46%), 10q (46%), 16q (46%), 19 (43%, both arms), 15q (35%), 6q (27%), 18q (19%), 10p (22%), 17p (41%), and 20q (22%). The minimal commonly lost regions in these chromosome arms were 1p36-pter, 1p31, 5q15-q23, 6q16, 6q24-qter, 8p12-p22, 8p23, 10cen-q21, 10q26, 10p11, 13q12, 13q21, 15cen-q21, 15q25-qter, 16q24, 17p, 18q22-qter, 19pter-q13.1, 20cen-q22, and 22q13.

The most frequently gained chromosome arms were (Figure 1) 8q (73%), 7q (43%), Xq (35%), 7p (32%), 18q (30%), 2q (27%), 3q (24%), Xp (24%), 11q (22%), 12q (22%), 13q (19%), 4q (19%), and 5q (14%). And the minimal commonly gained regions were 1q25-q32, 2q33, 3q25-q26, 4q13-q23, 5q14-q31, 7p15-p21, 7q21, 7q31, 8q21, 8q23-qter, 11q22, 12q21, 13q31, 18q12, Xpter-Xp21, and Xcen-q13. Figure 2 shows examples of different types of CGH findings of chromosome 8 in hormone-refractory prostate carcinomas.

### CGH Analysis of Primary-Recurrent Tumor Pairs

Table 1 summarizes the results of CGH analysis of the primary-recurrent prostate tumor pairs. On average, 51% of the genetic alterations found in the primary tumors were also found in the recurrent tumors. Three recurrent tumors showed more than 50% of the genetic aberrations found in the corresponding primary tumor. However, there was, for example, a case in which only 8% of the aberrations were shared by both tumors (case 1).

### FISH Analyses

To validate the CGH findings, we chose a subset of tumors for FISH analysis of caveolin (7q31), c-myc (8q24), bcl-2 (18q21.3), and AR (Xq12). These were the regions that were most commonly gained by CGH. Table 2 summarizes the comparison of CGH and FISH findings.

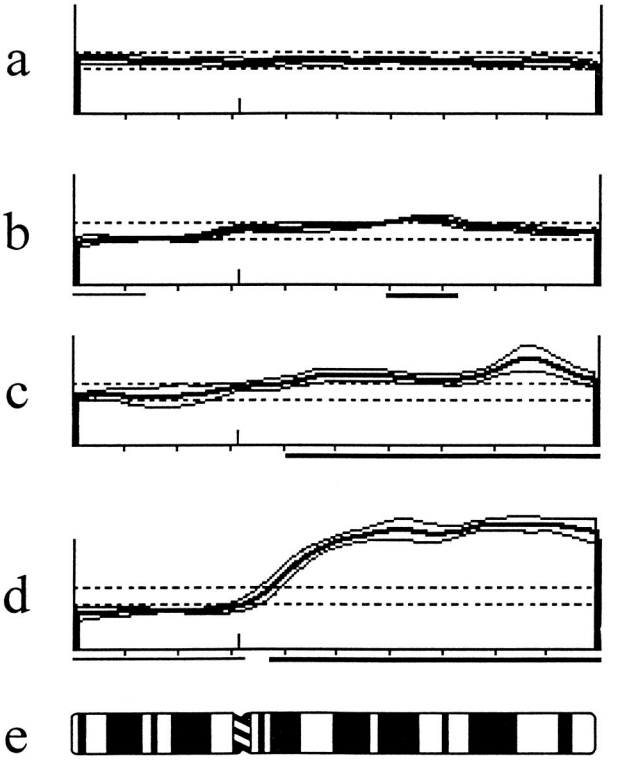


Figure 2. Mean green-to-red ratio profiles with ±1 SD from pter to qter obtained from CGH analysis of four locally recurrent hormone-refractory paraffin-embedded prostate tumors. The dotted lines represent ratio values of 0.85 and 1.15. a: Normal; b: Loss of 8p22-pter, gain of 8q21-q22; c: Gain of 8q12-qter with higher-level amplification at 8q24; d: Loss of 8p and gain of 8q12-qter; e: Chromosome 8 idiogram.

Table 1. Genetic Alterations in the Primary and Recurrent Tumor Pairs

| Case | Primary tumor | Recurrent tumor | Time to progression (months) |
|---|---|---|---|
| 1 | Gains: 22p, 22q<br>Losses: 1p, 1q, 2q, 4q, 5q, 6q, **8p**, 12q, 13q, Xq | Gains: 3q, 7p, 7q, 8q, 13q, Xq<br>Losses: **8p**, 19, 17, 20q | 15 |
| 2 | Gains: **7**, **8q**, 9p<br>Losses: **8p**, **13q** | Gains: **7**, **8q**<br>Losses: **8p**, **13q** | 36 |
| 3 | Gains: none<br>Losses: 1p, **6q**, **11q**, 12q, **13q**, X | Gains: 3q, 8q, 17q, Xp, Xq<br>Losses: **6q**, 8p, 10q, **11q**, **13q**, 15q, 18 | 59 |
| 4 | Gains: **8q**, 20p<br>Losses: 16p, 17 | Gains: **8q**, 4q, 5p<br>Losses: 6q | 46 |
| 5 | Gains: 2q, 3q, 5q, 7, **8q**, 11q, **12q**<br>Losses: **1p**, **8p**, **16p**, **17p**, **19**, **22** | Gains: **8q**, 10q, **12q**, 21q<br>Losses: **1p**, **8p**, 13q, 15q, **16p**, **17p**, 18q, **19**, **22** | 12 |
| 6 | Gains: 2p, **8q**, **16p**<br>Losses: **1p**, **2q**, **5p**, **5q**, **6q**, **8p**, **9p**, **13q**, **16q**, **18q** | Gains: **8q**, **16p**, Xq<br>Losses: **1p**, **2q**, 3, **5p**, **5q**, **6q**, **8p**, **9p**, **13q**, **16q**, **18q**, Xq | 22 |

The chromosome arms that contain either gains or losses are given. Genetic alterations that are shared by primary and recurrent tumors are in bold. The histological grades of the primary and the corresponding recurrent tumors were the same in each of the six sample pairs.

Altogether, 78% of cases showed similar results by CGH and FISH ($\kappa$ value of 0.57). Figure 2 shows recurrent tumors studied by FISH for c-myc, caveolin, and bcl-2. Seventeen of twenty-two tumors showed increased copy number of c-myc. Of the seventeen tumors, five (29%) showed high-level (more than three times more signals of c-myc *versus* the chromosome 8 centromere) amplification of c-myc with a mean ± SD copy number of signals (excluding the nuclei with only two signals) of 6.2 ± 0.9. Occasional nuclei with more than 10 copies of c-myc were also found. Four of eight cases showed equally increased copy number of the chromosome 7 centromere and 7q31 (caveolin) with a mean copy number of signals of 3.9 ± 0.6. And four of seventeen cases showed equally increased copy number of the chromosome 18 centromere and bcl-2, with a mean copy number of signals of 4.4 ± 1.3.

## Discussion

Here, we report results from the analyses of 37 recurrent hormone-refractory prostate carcinomas for genetic aberrations using CGH. The high number of alterations per tumors found by CGH emphasizes the genetic instability as an underlying mechanism of cancer development and progression. As compared with our previous CGH analysis of 31 unselected primary prostate carcinomas,[15] the recurrent tumors in this study contained almost four times more alterations than the primary tumors supporting our earlier findings that the clinical progression of prostate cancer is associated with genetic progression of the tumors.[8,24]

We analyzed also six primary-recurrent tumor pairs. Three tumor pairs (50%) showed a close genetic relationship as evidenced by a high number of shared genetic alterations in primary and recurrent tumors. For example, in case 6, 13/14 of the alterations (93%) found in the primary tumor were also present in the recurrent tumor (Table 1). The aberrations that were solely found in the recurrent tumors were losses of whole chromosome 3 and the telomeric part of Xq (Xq23-qter), as well as gain of Xcen-q13, indicating a relatively linear genetic progression of the tumor. The most likely target for the Xq gain in this tumor is the AR gene, which was found to be fivefold amplified in the specimen.[8] On the other hand, in case 1, only 1 of the 12 genetic aberrations found in the

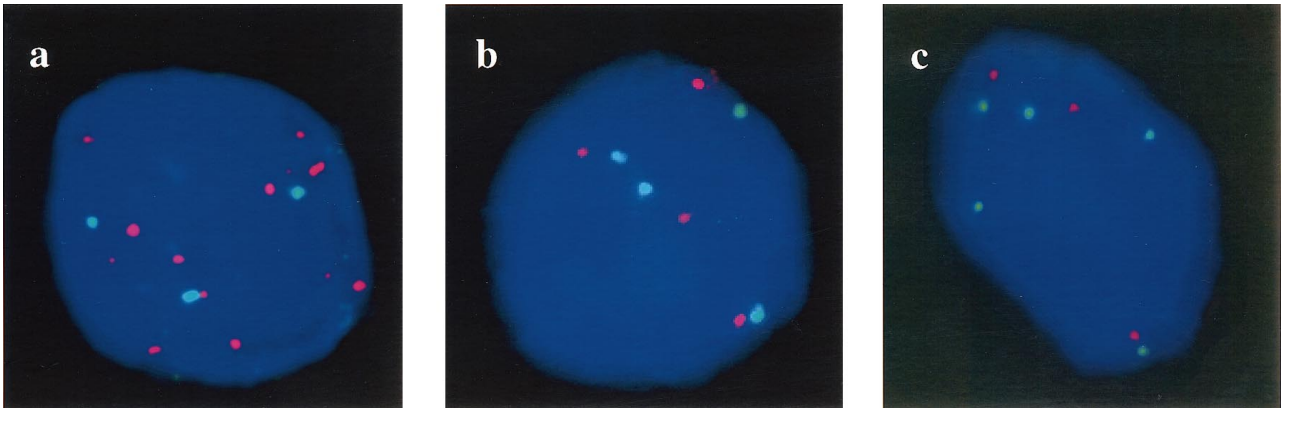


Figure 3. Interphase FISH analyses of hormone-refractory prostate cancer with probes for chromosome 8 centromere (in green) and c-myc (in red) (a), chromosome 7 centromere (in red) and caveolin (in green) (b), and chromosome 18 centromere (in red) and bcl-2 (in green) (c). a: Multiple copies of c-myc and three copies of centromere are seen. b: Four copies of centromere and caveolin are seen. c: Three copies of centromere and five copies of bcl-2 are found. Nuclei are counterstained with DAPI.

Table 2. Condordance between CGH and FISH Data for the 7q31 (Caveolin), 8q24 (c-myc), 18q21.3 (bcl-2), and Xq12 (AR) Regions

| | FISH | |
|---|---|---|
| CGH | Aberrant | Normal |
| Aberrant | 31 | 2 |
| Normal | 15 | 29 |

The $\kappa$ value was 0.57.

primary tumor were present in the recurrent counterpart. Prostate cancer is commonly considered to be heterogeneous disease. Studies on the whole-mount prostatectomy specimens have shown that prostate gland may contain several carcinoma foci, which appear not always to be physically in contact with each other. These foci may contain different genetic changes.[25-27] Jenkins and co-authors[27] have also shown that in some cases it is the minor, instead of the major, primary carcinoma focus that metastasizes. Still, the significance of such multifocality of prostate cancer is inadequately known. The results in this study suggest that the androgen ablation therapy acts as a strong selection force in a similar fashion as a metastases event.[20,27] In some cases, it may select for a clone that has acquired only a few additional genetic alterations, such as AR gene amplification. In other cases, the treatment may select for a genetically very different clone or a completely different clone than the primary tumor, suggesting that there are several different mechanisms that may underlie the progression of prostate cancer during endocrine treatment. The fact that recurrent tumors may share only a few genetic alterations with primary tumors indicates also that biomarkers measured exclusively from primary tumors give only a restricted view on the biological properties of hormone-refractory prostate cancer.

In this study, every chromosome arm showed genetic alterations in at least one tumor by CGH, probably as a result of random genetic instability. However, some of the chromosomal regions were often altered, suggesting that these regions contain oncogenes and tumor suppressor genes (TSGs) that are important in the development and progression of prostate cancer. The most commonly lost regions were 8p, 13q, 1p, 10q, 17q, 19, and 22, whereas the most commonly gained regions were 8q, 7q, Xq, 18q, and 7p. Most of these regions had been implicated in prostate cancer earlier by either CGH or loss of heterozygosity (LOH) studies.[13–16,28–30]

Three chromosome arms that have most extensively been studied in prostate cancer, due to the high frequency of LOH found in these regions, are 8p, 10q, and 16q. Loss of 8p as detected either by LOH, CGH, or FISH may well be the most common genetic alterations in prostate cancer.[13–16,30–32] It also seems to be an early event in tumorigenesis, as prostate intraepithelial neoplasias have also shown LOH at 8p.[33] In this study, we found two minimal commonly deleted regions, 8p23 and 8p12-p22, suggesting that there are several still unknown target genes for the deletions in 8p.

Deletion of 10q was found in more than 40% of tumors. Chromosome 10q contains two candidate prostate cancer tumor suppressor genes: MXI1 at 10q25[34] and the recently cloned PTEN at 10q23.[35,36] Neither one of these genes map to the minimal commonly deleted regions (10cen-q21 and 10q26) found in this study, suggesting existence of yet another TSG in 10q. We have not commonly found losses at 10q in primary prostate carcinomas,[15] indicating that the inactivation of the TSGs in 10q may be a late event in the development of prostate cancer.

As in this study, LOH and deletions at 16q have been found in approximately 50% of prostate carcinomas.[15,16,28–30] One candidate TSG in chromosome 16 is E-cadherin (16q22.1), the decreased expression of which has been found in poorly differentiated and clinically aggressive prostate carcinomas.[37–39] However, no mutations have, so far, been identified in the coding region of the gene. We and others have also found that the minimal commonly lost region is 16q24,[16,40] suggesting that 16q may harbor TSGs other than E-cadherin.

Yet another frequently deleted chromosome region in prostate cancer is 13q.[15,16,30] Chromosome 13 contains at least three putative TSGs: Rb1 (13q14), BRCA2 (13q12-q13), and BRUSH-1 (13q12-q13). Although reduced expression of Rb has been found in prostate carcinomas, only few prostate tumors have shown mutations in the coding region of the gene.[41] Here, we found two minimal commonly deleted chromosomal regions: 13q12 and 13q21. Surprisingly, also gains of 13q, which has not previously been reported in prostate cancer, except *in vitro*,[42] was found in 20% of the tumors.

Two chromosomes that were frequently lost, but have not been previously implicated in prostate cancer by either LOH or CGH studies, were 1p and 19. Early studies suggested that these two regions may show artificial deletions by CGH as evidenced by alterations in 1p and 19 even in normal-normal control hybridization. However, these problems are believed to be caused by differential hybridization of biotin and digoxigenin-labeled DNAs. The use of directly fluorochrome-conjugated nucleotides, as in this study, has abolished this phenomenon.[12] Also, the negative controls (DNA from either paraffin-embedded blocks or blood lymphocytes) showed no variations in 1p or 19. Thus, we believe that the findings are true. Chromosome 19 contains some putative TSGs, such as BAX[43] and LKB1,[44] whereas the p53-related putative neuroblastoma TSG was recently mapped to 1p36.[45] However, the role of these TSGs in prostate cancer has not been studied so far.

Before CGH, there were no effective ways to screen the genome for gains of DNA sequences. Therefore, CGH has probably contributed most to the screening of tumors for amplifications.[10–12] CGH studies have already indicated that primary prostate cancers contain very few gains or high-level amplifications.[13,15] On the other hand, as in this study, hormone-refractory recurrent tumors commonly carry such gains. This is consistent with the notion that oncogene amplification is often a rather late event in tumor progression, affecting cells that are unstable enough to be able to amplify DNA.[46,47] In this study,

the most common genetic alteration, together with the loss of 8p, was the gain of 8q. The 8q gain in prostate cancer was first described by Bova and co-workers[31] using Southern analysis. Subsequently, it has been shown that 8q gain is common in hormone-refractory[15] as well as in metastatic lesions of prostate cancer.[16] Recurrent tumors exhibit 8q gain >10 times more often than the primary tumors.[15]The presence of extra copies of chromosome 8 centromere is reported to be associated with a short progression-free interval,[48] and amplification of 8q24 region has been shown to be associated with the presence of lymph node metastases.[49] In addition to prostate cancer, the 8q gain has been found by CGH, for example, in breast and bladder cancer.[50,51] And, in breast cancer, the 8q gain seems to be associated with poor prognosis.[52]

CGH analysis, here and earlier,[15,16] has identified two minimal commonly amplified regions: 8q21 and 8q23-q24. These findings suggest that there may well be several target genes for the gain of 8q. A natural candidate target gene for the 8q24 amplification is c-myc, which plays significant roles in the regulation of cellular proliferation, differentiation, and apoptosis.[53] Overexpression of c-myc has been detected in prostate cancer.[54,55] Still, until recently, amplification of c-myc had not been found in prostate carcinomas *in vivo*.[27] In this study, a subset of tumors with 8q gain showed high-level amplification of c-myc. However, based on these data, we cannot exclude the possibility that the real target gene for 8q24 amplification could also be some gene other than c-myc.

Other frequently gained chromosomal regions were 7q, 7p, Xq, and 18q. Increased copy number of chromosome 7 has been detected by FISH,[48,56,57] and trisomy 7 may be associated with progression of disease.[48,56] In several tumors, the entire chromosome 7 was gained, but we were able to narrow down three separate regions of minimal gains: 7p21-p15, 7q21, and 7q31. We applied also locus-specific (caveolin; 7q31) and centromeric FISH to evaluate the CGH findings. Equally increased copy number of centromere and caveolin was commonly found, whereas there were no high-level amplifications on the 7q31 region. Chromosome 7 contains several known genes, such as CLK2, MDR1, and elongation factor-1-γ, of which altered function could theoretically be involved in the progression of prostate cancer.

Gain of Xq was found in one-third of the cases. We have already earlier shown that the most likely target gene for Xq amplification is the AR gene. It is highly amplified and overexpressed in approximately 30% of hormone-refractory tumors.[8,9] Also, 18q was gained in approximately one-third of the tumors. Bcl-2 oncogene, which has commonly been found to be overexpressed in recurrent prostate cancers,[3–5] is located in 18q21.3. Although bcl-2 is located outside the minimal commonly gained regions (18q12) according to CGH, we decided to analyze the exact copy number of bcl-2 by FISH. Four cases showed increased copy number of bcl-2; however, no high-level amplifications were found. Thus, it seems that bcl-2 overexpression in prostate cancer is not due to the high-level amplification of the gene.

The ability to use DNA extracted from paraffin-embedded tumor blocks for CGH has made it possible to obtain samples from vast archives of routine pathology laboratories. It has been shown that the quality of CGH is almost equally good whether DNA from paraffin-embedded tumor blocks or from frozen tumor samples are used.[22] For example, the SDs of the mean fluorescence intensity ratio profiles, which are used to indicate the quality of CGH hybridizations, were practically equally as low in the CGH analysis of paraffin-embedded samples as we have found in the analysis of the frozen samples. Indeed, several studies using paraffin-embedded blocks for CGH have already been published.[58–60] We also used FISH to validate the CGH findings. In 78% of cases, FISH and CGH results were in agreement. However, some of the AR gene amplifications were undetected by CGH, whereas increased copy number of 7q31, 8q24, and 18q21.3 were found almost equally well by CGH and FISH (data not shown). This could be due to the small size of the AR gene amplicon as compared with the size of gained regions at 7q, 8q, and 18q.

In conclusion, the comparison of genetic alterations in the primary-recurrent tumor pairs revealed strong genetic heterogeneity and a nonlinear relationship of the tumors. The hormone-refractory recurrent prostate carcinomas contained a high number of both gains and losses of DNA sequences. Especially, the gain of 8q seemed to be associated with progression of the disease. Identification of target genes for the 8q gain would, therefore, most likely reveal important mechanisms of tumor progression.

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