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Lead article

Resolution of genotypic heterogeneity in prostate tumors using polymerase chain reaction and comparative genomic hybridization on microdissected carcinoma and prostatic intraepithelial neoplasia foci Ben Beheshti^{a,b}, Bisera Vukovic^{b,c}, Paula Marrano^c, Jeremy A. Squire^{a,b,c,*}, Paul C. Park^{a,b}

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Abstract

Prostate cancer (CaP) is a multifocal heterogenous disease. A major challenge in CaP research is to identify genetic biomarkers that herald aggressive transformation. To investigate the effect of tumor heterogeneity on the analysis of genomic aberration, we compared the results of comparative genomic hybridization (CGH) analysis of DNA extracted from tumor bulk against that of DNA amplified by degenerate oligonucleotide primed polymerase chain reaction (DOP-PCR) from homogeneous cell population obtained by laser capture microdissection of discrete tumor foci. Sampling by microdissection, aberrations were observed in three of three foci of carcinoma involved with prostatic capsule, and in two of three prostatic intraepithelial neoplasia (PIN) foci examined. Carcinoma foci consistently exhibited more extensive aberrations than the PIN samples obtained from the same tumor. Within these samples, the different tumor foci exhibited gain of 8q, whereas PIN showed no consistent aberration. Using bulk extracted DNA, CGH detected aberrations in only 3 of 21 samples investigated, despite the known trisomy 8 status, as revealed by fluorescence in situ hybridization. The results of this study demonstrate that CGH analysis using bulk dissected fresh tissue is insufficiently sensitive to fully detect the chromosomal numerical aberrations in CaP. Given the considerable intratumor genomic heterogeneity, CGH with microdissection and DOP-PCR amplification provides a more complete repertoire of aberrations as well as a better phenotype-genotype correlation in prostate tumors. © 2002 Elsevier Science Inc. All rights reserved.

1. Introduction

In North America, prostate cancer (CaP) is the leading cancer incidence in men and the second most common cause of male cancer mortality [1]. Our understanding of the molecular genetic changes that underlie the progression of this disease remains at an early stage, as CaP exhibits both inter- and intratumor genotypic and phenotypic heterogeneity that complicates molecular and histopathological assessment and outcome prediction [2–5].

Prostate cancer is characterized by multifocal presentation [6]. Consistent with this idea, it was recently shown that a much greater frequency of chromosomal aberrations can be detected if microdissection and specialized culture methods are utilized [7]. A newly diagnosed man with CaP will have an average of five apparently independent lesions [8]. In addition, there is growing evidence [5,9–11] that both cancerous and pre-cancerous lesions within a given prostate tissue are non-clonal, further indicating the multifocal nature of this disease.

Currently, the histopathological assessment of CaP is based on the Gleason system, which assigns a clinical grade to a given tumor based on the most prominent and representative histological features. This system not only has a significant clinical impact in predicting the outcome of the disease, but much of the current research effort in CaP is also directed towards identifying the molecular changes as a function of the Gleason grade. In this study, we used the genome-wide scanning technique of CGH in combination with degenerate oligonucleotide primed polymerase chain reaction (DOP-PCR) and laser capture microdissection (LCM) to demonstrate the genotypic heterogeneity among distinct foci within tumor samples, and to identify the karyotypic changes asso-

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ciated with the prostatic capsule invasion. Furthermore, we evaluated the effectiveness of this technique against the traditional CGH method, using bulk-extracted DNA, in identifying the chromosomal gains and losses in early-stage CaP specimens. In addition, we employed fluorescence in situ hybridization (FISH) technique to investigate the genotypic heterogeneity as a feature of early-stage (pT1-T2) CaP.

2. Materials and methods

2.1. Tissue accrual and sample preparations

All samples utilized for this study were from patients undergoing radical prostatectomy at the University Health Network and presenting with evidence of high tumor volume and no history of radiation or chemotherapy. A small wedge (approximately $1-2 \text{ cm}^3$) of tumor tissue was dissected from the excised prostate. Criteria for inclusion in this study were based on the presence of a widely ranging variation in the Gleason pattern, as determined by frozen section.

The obtained tissue sample was processed following two different protocols. In one set of experiments (21 patient samples), the tissue was bisected, and one of the pieces was used to establish a short-term tissue culture (see below) for interphase FISH analysis. The remaining piece was placed in DNA extraction buffer and the genomic DNA from the tumor bulk extracted following standard protocols [12,13].

In a second set of experiments (three patient samples: A, B, and C), the tissue was immediately fixed in 70% ethanol (4°C, overnight) and embedded in paraffin. Serial sections, 10 μ m in thickness, were obtained from the paraffin-embedded tissue and stained with H & E. Homogeneous populations of epithelial cells were obtained from the sections using laser capture microdissection system (Arcturus, Mountain View, CA, USA), from two different regions in each tumor sample, including: (1) acini of epithelial cells of Gleason pattern 3, which have infiltrated the prostatic capsule, and (2) acini of high grade prostatic intraepithelial neoplasia (PIN) situated nearby (<3 mm) which were not associated with the capsule. Typically, 200–400 cells were collected from the serial sections for each region, and processed for genome amplification by DOP-PCR.

2.2. Amplification of the genomic DNA by DOP-PCR

The dissected cells were incubated in 20 μ L of digestion buffer (0.1% SDS, 1 μ g/ μ L proteinase κ in Tris-HCl, pH 8.0) overnight. Following incubation at 90°C to inactivate the proteinase, the product containing the genomic DNA was serially diluted tenfold to concentrations of 1:10, 1:100, and 1:1000 of the original solution. One microlitre of each dilution was used as the template in separate PCR reactions.

The PCR amplification and labeling of the probe were carried out in three steps, using the Clontech cDNA PCR enzyme mix (Clontech, Palo Alto, CA, USA). In the first step, 0.1 μ L of dNTP (10 mM), 0.5 μ L of the primer (5'-CCGACTC GAGNNNNNNATGTGGG-3',10 μ M), 0.5 μ L of PCR buffer

 $(10\times; \text{Clontech})$, and 0.2 μ L of enzyme mix (Clontech) were added to the template DNA, and the volume adjusted to 5 µL. In addition, a series of reaction mixtures containing 30 ng, 3 ng, or 300 pg of normal male DNA, obtained from human spleen tissue, were prepared in parallel for generation of reference probes for CGH. The reaction mixtures were denatured at 95°C for 5 minutes, and carried through eight cycles of denaturation (94°C, 1 minute), annealing (30°C, 1 minute) and extension (72°C, 3 minutes). Following a final extension of 10 minutes at 72°C, each reaction mixture was supplemented with 20 µL of reaction mixture containing 0.6 µL of dNTP (10 mM), 1.25 µL of primer (25 µM), 2.5 µL of PCR buffer, and 0.5 µL of the enzyme. The reaction mixture was denatured at 95°C for 5 minutes, and further cycled through 30 rounds of denaturation (94°C, 1 minute), annealing (56°C, 1 minute) and extension (72°C, 3 minutes), followed by a final extension of 10 minutes at 72°C. The product was purified by column chromatography (Qiaquick PCR Purification kit; Qiagen, Mississauga, Ontario, Canada) and the amplification verified by electrophoresis on 1% agarose gel. Typically, the optimal product, as determined by the largest product size, was obtained from the initial template of 3 ng or 300 pg of normal male DNA, or from the tenfold or the hundredfold dilutions of the digest of the microdissected sample.

2.3 Probe preparation

For labeling of the PCR amplified DNA, 4 μ L of the selected product was added to 46 μ L of reaction mixture containing 1 μ L of dNTP (10 mM), 2.4 μ L of primer (25 μ M), 5 μ L of PCR buffer, 1 μ L of the enzyme, and either 2 μ L of 0.4 mM biotin-14dATP (tumor DNA) or 0.8 μ L of 1 mM digoxigenin-11dUTP (normal reference DNA). The labeled probe was generated by 16 rounds of amplification using the parameters specified in the second step. The final product was purified by column chromatography, quantified by spectroscopy, and sized by gel electrophoresis.

Alternatively, 2 μ g each of normal and bulk-extracted tumor DNA were labeled by nick translation with digoxigenin-11 dUTP (Roche, Basel, Switzerland) and biotin-14 dATP (GIBCO BRL, Burlington, Ontario, Canada) respectively, as previously described [14,15]. All final labeled probes ranged between 500 bp and 2 kb in size.

2.4. Comparative genomic hybridization (CGH)

Comparative genomic hybridization was performed as previously described [14,15]. Ten metaphases were analyzed to create the final CGH profile with 99% confidence intervals. Negative controls in which normal DNA was compared to itself, and positive controls using IMR32 neuroblastoma cell line, which has been previously characterized in our laboratory, were routinely included in the experiments. In addition, controls in which the DOP-PCR–amplified normal DNA was compared to nick-translated normal DNA were employed to ensure that the DOP-PCR amplification did not introduce artefactual gains or losses in the results.

2.5. Short-term tissue culture

For 15 of 21 samples in which the DNA was bulkextracted, tissue cultures were prepared and maintained for short term (<1 week) for interphase FISH analysis. For this, the tissue sample was dissociated into small pieces and digested in 250 U/ml collagenase IV (GIBCO BRL) in culture media (RPMI-1640, 10% fetal bovine serum, antibiotics) for 2–3 hours. Resulting cell suspension was centrifuged gently and washed with phosphate buffer saline, seeded into tissue culture flasks for attachment (usually 1–3 days) and subsequently harvested for interphase FISH analysis.

2.6. FISH

Harvested cytogenetic preparations from patients were dropped onto glass slides as previously described [16] using 1.5 hour Colcemid treatment and 75 mM KCl hypotonic treatment. Normal cytogenetic control slides were made from phytohemagglutinin-stimulated normal male lymphoblasts. Additionally, 5- μ m sections were cut from the paraffinembedded tissue (patient C) for confirmatory FISH analysis, with a corresponding H & E slide for confirmation of location of tumor cells. Denaturation of the centromere enumeration probe 8 (CEP8) and 8q24 (*MYCC*) FISH probes (Vysis, Downers Grove, IL, USA) and application of the probes to the slides were as per manufacturer's instructions (Vysis) and previously reported [6]. At least 100 nuclei were used for enumerating the cohybridized probes for each sample.

3. Results

3.1. CGH analysis of bulk-extracted tumor

CGH analysis of the bulk-extracted tumor samples revealed no chromosomal imbalances in 16 of 21 samples examined. In two of five samples which showed copy number changes, two were considered inconclusive, given that the only changes observed were associated with the large heterochromatic region of the Y chromosome (Yq12), which is an established cytogenetic polymorphism. In contrast, three patient samples, namely patients CaP13, CaP14, and CaP26, revealed a gain of the long arm of chromosome 8 (8q). Furthermore, a concurrent loss of the short arm of chromosome 8 (8p) was observed in CaP13 and CaP14, suggestive of isochromosome 8q formation. Both CaP13 and CaP14 also showed loss of 13q, while the latter showed additional loss of 16q and 18q (Fig. 1). The positive control IMR32 neuroblastoma line prepared for CGH showed highlevel amplification at the 2p22 and 2p24 chromosomal regions, as expected (not shown). The negative control (normal male DNA) showed no CGH imbalance, as expected.

3.2. CGH analysis of microdissected DOP-PCR-derived tumor

In contrast, extensive chromosomal gains and losses were observed by CGH in two of three microdissected samples of PIN foci, as well as in three of the three carcinoma foci infiltrating into the capsule (Fig. 2). The control experiment that compared the PCR-amplified normal DNA with the nick-translated normal DNA showed no chromosomal imbalances (not shown), suggesting that the changes observed in the microdissected samples were not artifacts of the PCR amplification. Within a given tumor specimen, the carcinoma foci consistently exhibited a more complex pattern of changes than the corresponding PIN (Table 1). Moreover, in two of the three tissue samples analyzed, a subset of the changes observed in PIN was also represented in the adjacent carcinoma sample from the same patient (Table 1). This occurrence of common genomic imbalances in both PIN and carcinoma provides support for the view that the PIN may be a precursor lesion of carcinoma [6,17]. A gain of 8q was a consistent feature observed among the carcinoma foci from all three patients, while two of the three samples also consistently exhibited $+13q14.3 \sim 21.2$ and -16p (Fig. 2, Table 1). Paraffin FISH using MYCC probe (8q24) was used to confirm the finding of 8q gain (Fig. 3). No consistent pattern of changes was noted among the PIN samples.

3.3. Cellular heterogeneity of chromosome 8 determined by interphase FISH (I-FISH)

Dual-color I-FISH was used to examine tumor preparations to determine the extent of cellular heterogeneity for chromosome 8 copy number alteration (Fig. 1). Centromere 8 probe (CEP8, green) was used together with MYCC (8q24, orange) probe to interrogate the extent of 8q gain in the patient samples (Table 2). MYCC was found to always correlate in a 1:1 ratio with CEP8 in the normal control and the patient material nuclei. In control normal male lymphocytes, trisomy 8, as determined by 3 CEP8/MYCC signals, was observed in less than 1% of the cells. In contrast to this baseline frequency, all the patient samples for which cytogenetic preparations were established, including those in which CGH failed to identify a gain of chromosome 8, exhibited a frequency of trisomy ranging from 5-44% (Table 2). In addition, low levels of monosomy and polysomy of chromosome 8 were also detected in all patient samples (Table 2).

4. Discussion

There is mounting evidence that CaP is a multifocal, heterogeneous disease. Studies examining allelic imbalances reveal that tumor foci within a given prostate are genotypically heterogenous [5,9,10,18]. Moreover, similar studies of PIN indicate heterogeneity exists at early stage of tumor progression, indicating that several foci of carcinoma may arise independently within a given tumor [10,17].

In this study, CGH analysis using bulk-extracted DNA detected significant aberrations in only three of the 21 tumor samples examined. This figure is significantly lower than the previous CGH report of aberrations in 74% of the studied samples [19]. This difference may be a result, in part,



Fig. 1. Representative metaphase spreads analyzed by CGH, using bulk-extracted DNA from patients CaP14 and CaP10 (A and D respectively), and corresponding ideogram (B and E). Regions of gains and losses in tumor DNA are represented as shifts to higher and lower green-to-red ratios, respectively. Note the lack of 8q gain in the CGH profile of CaP10 (E), despite the evidence of high frequency of trisomy 8, as detected FISH analysis using CEP8 (green) and *MYCC* (8q24; orange) probes (C and F).

of our analysis focusing on significantly earlier stages of the disease compared to those previously published [19–22]. Our FISH analysis showed considerable cell-by-cell heterogeneity at the CEP8/*MYCC* loci in all 15 cytogenetic preparations. It is particularly interesting that in the patient sample that exhibited the most pronounced degree of trisomy 8 (44%; CaP10), the corresponding CGH analysis failed to detect a gain (Fig. 1). The poor correlation between imbalances detected by CGH analysis of bulk-extracted DNA and the parallel cell-by-cell analysis by FISH is an indication that this technique is not adequate for detecting the heterogeneous changes of CaP.

Recently, several investigators have reported a significant advantage in combining CGH with the techniques of DOP-PCR and LCM in identifying the aberrations in prostate and other tumor types [11,23–31]. With this approach, Kim et al. have reported positive identification of aberrations in 100% of the tumor samples they screened [30]. This figure is consistent with the data presented herein, where all three of three tumor foci and two of three PIN foci exhibited positive aberrations. It is also noteworthy that a subset of these aberrations was uniquely present in only one of the pair of foci obtained from the same tissue sample, and would likely have gone undetected if the tissue were sampled as a whole using bulk extraction methods. Therefore, the complete repertoire of genomic aberrations in a given tumor is better represented by the sum of the changes in individual foci, rather than the averaged profiles indicated by the conventional CGH. Moreover, analysis at the level of individual foci provides a better correlation of the genomic changes with the phenotypic features.



Fig. 2. H & E images and corresponding CGH analysis of focus of carcinoma (A and B) and PIN (C and D), microdissected by laser capture from the same representative tissue sample (patient C). Note the extension of the carcinoma into the capsular margin, demarcated by a large blood vessel (arrow, A). (E) summarizes the gains (shown right of the chromosome) and losses (left of the chromosome) in the carcinoma (red) and PIN (blue) foci from this and two other patient samples.

Allelotyping experiments have demonstrated frequent involvement of chromosome 8 in CaP tumorigenesis [5,32-35]. Recent studies by Macoska et al. [36] and Virgin et al. [37] using human papillomavirus (HPV) E6/E7 and simian virus 40 (SV40) large T antigen immortalized CaP patient cell lines, showed a direct correlation between 8p loss of heterozygosity (LOH) allelotyping data and isochromosome 8q formation or other structural rearrangements of 8p by molecular cytogenetics. Alers et al. [38] demonstrated by FISH in localized prostate tumors, lymph node metastases, and distant metastases samples that +8 was more frequent than -8. Subsequent examination of the lymph node metastasis sample allowed correlation of +8 in interphase FISH with 8q gain as determined by CGH, and, conversely, -8 by FISH with 8p loss by CGH [38]. Together, these observations suggest that 8q gain may be independent of and contributes to the 8p- genotype in the tumorigenic process, but can also sometimes occur through isochromsome 8q formation. This view is consistent with the data presented herein, in which

two of three patients (CaP13, CaP14) examined by CGH using bulk-extracted DNA exhibited 8q gain and 8p loss concomitantly (Fig. 1). In contrast, CGH analysis of all the microdissected specimens revealed a gain of 8q, in absence of 8p loss, to be a consistent feature of the invasive foci. This apparent discrepancy may be reconciled by the possibility that the 8p loss may be a recurrent aberration represented in several independent microfoci. This loss may be sufficient for detection by conventional CGH but appears to be irrelevant to the development of the invasive phenotype.

There is evidence that suggests that these foci may be genomically unstable, through microsatellite [39–42] and/or chromosomal instability [6,17,39,40,43–45], giving rise to further variant foci during tumor progression. Genomic instability may be genotypically expressed as microsatellite instability as a result of failing DNA repair at the nucleotide level leading to replication errors, or as chromosomal instability due to aberrations in the mitotic machinery leading to chromosomal copy number and structural changes, that ultiTable 1 Summary of aberrations in microdissected foci of carcinoma and PIN from three patients

Patient	Gleason pattern 3	PIN
A	+3 +4q13.3 +4q23 \sim q24 +8q +13q14.3 \sim q21.1 +14q13 \sim q22.3 -16p -17p	$+3p24\sim pter +3p12.2\sim q13.2 +8q +15q15\sim q21.2 -20$
В	$+ xq + 2q31.1 \sim q31.2 + 5q11.1 \sim q12 + 8q + 10q21.1 \sim q22.2 + 13q14.2 \sim q21.2 - 16 + Xq - Y$	$\begin{array}{c} +2q24.1 \sim q31.2 \\ +8q21.3 \sim q22.3 \\ +Xq21.3 \sim q22.2 \\ -Y \end{array}$
С	+8q12.1~qter +Xp21.3~pter	

mately leads to aneuploidy and destabilization of the tumor karyotype. Given the slow rate of tumor growth in CaP, it may be speculated that the disease progression from early lesions, including PIN, involves independent evolution of several individual foci that have acquired different genotypic changes in response to the selective pressure of the microenvironment of the prostate [46,47]. In this regard, it is feasible that the repertoire of the individual foci within a tumor, rather than the most representative feature, may have a greater impact on the stage and outcome of the disease than the Gleason score, per se. Thus, the genomic profile of copy number imbalance in each tumor focus, and in general in the tumor itself, likely represents a unique endpoint of a complex interplay between genomic divergence through the multistep accumula-



Fig. 3. I-FISH analysis of chromosome 8 copy number gain in CaP from patient C using *MYCC* probe (white signals) on a 5- μ m paraffin section from a typical region of normal prostate epithelial (A) and carcinoma (B) cells from the same representative tissue sample. Histopathology was correlated with corresponding H & E tissue section (not shown). Note the gain of *MYCC* copy number in carcinoma versus normal cells.

,	Table 2
	I-FISH analyses of the centromere 8/MYCC copy number in the CaP
1	patients

	Chromosome 8 copy number				
Case no.	1 1%	2 96%	3 0%	≥4 3%	
Normal lymphocytes					
1	4	84	6	6	
2	7	76	5	12	
3	1	90	7	2	
4	1	86	7	6	
5	5	76	18	1	
6	5	80	8	7	
7	1	85	11	3	
8	10	80	7	3	
9	5	86	8	1	
10	4	49	44	3	
11	10	83	4	3	
12	3	90	6	1	
13	8	70	14	8	
14	2	92	6	0	
15	3	92	4	1	

In each case, the number of *MYCC* signals equaled that of the centromere 8 signals. Note the variability in the centromere 8 copy number around the diploid modal value, and the significantly greater frequency of trisomy in all of the patient samples compared to the baseline frequency observed in the normal lymphocyte. Boldface indicates interesting chromosome 8 copy changes observed by this method.

tion of genetic changes [5,32,34,35,45,48–51], and convergence through selection. Overall, these observations, taken together with the present data, further support the notion of independent evolution of individual microfoci within tumors, and further suggest that the disease progression may be impacted by the individual, unique evolution of each foci rather than the tumor as a whole.

In conclusion, the present study demonstrates that CGH analysis using bulk dissected fresh tissue is not sufficiently sensitive to fully detect the chromosomal numerical aberrations in CaP. Given the considerable intratumor genomic heterogeneity, CGH in conjunction with microdissection and DOP-PCR amplification provides a more complete repertoire of aberrations as well as a better phenotype-genotype correlation in prostate tumors.

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References

- Haas GP, Sakr WA. Epidemiology of prostate cancer. CA Cancer J Clin 1997;47:273–87.
- [2] Wullich B, Breitkreuz T, Zwergel T, Unteregger G, Seitz G, Zang KD. Cytogenetic evidence of intratumoral focal heterogeneity in prostatic carcinomas. Urologia Internationalis 1992;48:372–7.
- [3] Micale MA, Sanford JS, Powell IJ, Sakr WA, Wolman SR. Defining the extent and nature of cytogenetic events in prostatic adenocarcinoma: paraffin FISH vs. metaphase analysis. Cancer Genet Cytogenet 1993;69:7–12.
- [4] Aihara M, Wheeler TM, Ohori M, Scardino PT. Heterogeneity of prostate cancer in radical prostatectomy specimens. Urology 1994;43:60–6.
- [5] Macintosh CA, Stower M, Reid N, Maitland NJ. Precise microdissection of human prostate cancers reveals genotypic heterogeneity. Cancer Res 1998;58:23–8.
- [6] Al-Maghrabi J, Vorobyova L, Chapman W, Jewett M, Zielenska M, Squire JA. p53 alteration and chromosomal instability in prostatic high-grade intraepithelial neoplasia and concurrent carcinoma: analysis by immunohistochemistry, interphase in situ hybridization, and sequencing of laser-captured microdissected specimens. Mod Pathol 2001;14:1252–62.
- [7] Teixeira MR, Waehre H, Lothe RA, Stenwig AE, Pandis N, Giercksky KE, Heim S. High frequency of clonal chromosome abnormalities in prostatic neoplasms sampled by prostatectomy or ultrasoundguided needle biopsy. Genes Chromosomes Cancer 2000;28:211–9.
- [8] Bastacky SI, Wojno KJ, Walsh PC, Carmichael MJ, Epstein JI. Pathological features of hereditary prostate cancer. J Urol 1995;153:987–92.
- [9] Fuijter ET, Miller GJ, van de Kaa CA, van Bokhoven A, Bussemakers MJ, Debruyne FM, Ruiter DJ, Schalken JA. Molecular analysis of multifocal prostate cancer lesions. J Pathol 1999;188:271–7.
- [10] Bostwick DG, Shan A, Qian J, Darson M, Maihle NJ, Jenkins RB, Cheng L. Independent origin of multiple foci of prostatic intraepithelial neoplasia: comparison with matched foci or prostate carcinoma. Cancer 1998;183:1995–2002.
- [11] Zitzelsberger H, Kulka U, Lehmann L, Walch A, Smida J, Aubele M, Lorch T, Hofler H, Bauchinger M, Werner M. Genetic heterogeneity in a prostatic carcinoma and associated prostatic intraepithelial neoplasia as demonstrated by combined use of laser-microdissection, degenerate oligonucleotide primed PCR and comparative genomic hybridization. Virchows Arch 1998;433:297–304.
- [12] Ausubel F, Brent R, Kingston R, Moore D, Seidman J, Smith J, Sturhl K. Current protocols in molecular biology. New York: John Wiley & Sons, Inc., 1998.
- [13] Dracopoli NC. Current protocols in human genetics. New York: John Wiley & Sons, Inc., 1998.
- [14] Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F, Pinkel D. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 1992;258:818–21.
- [15] Kallioniemi OP, Kallioniemi A, Piper J, Isola J, Waldman FM, Gray JW, Pinkel D. Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. Genes Chromosomes Cancer 1994;10:231–43.
- [16] Gustashaw KM. Chromosome stains. In: Barch MJ, Knutsen T, Spurbeck JL, editors, The AGT cytogenetics laboratory manual, 3rd ed. Philadelphia: Lippincott-Raven Publishers, 1997, pp. 259–324.
- [17] Al-Maghrabi J, Vorobyova L, Toi A, Chapman W, Zielenska M, Squire JA. Identification of numerical chromosomal changes detected by interphase FISH in high-grade prostate intraepithelial neoplasia (HPIN) as a predictor of carcinoma. Arch Pathol Lab Med 2001;126: 165–9.
- [18] Takimoto Y, Shimazui T, Akaza H, Sato N, Noguchi M. Genetic heterogeneity of surgically resected prostate carcinomas and their biopsy specimens is related to their histologic differentiation. Cancer 2001; 91:362–70.
- [19] Visakorpi T, Kallioniemi AH, Syvanen AC, Hyytinen ER, Karhu R, Tammela T, Isola JJ, Kallioniemi OP. Genetic changes in primary

and recurrent prostate cancer by comparative genomic hybridization. Cancer Res 1995;55:342–7.

- [20] Cher ML, Bova GS, Moore DH, Small EJ, Carroll PR, Pin SS, Epstein JI, Isaacs WB, Jensen RH. Genetic alterations in untreated metastases and androgen-independent prostate cancer detected by comparative genomic hybridization and allelotyping. Cancer Res 1996; 56:3091–102.
- [21] Cher ML, MacGrogan D, Bookstein R, Brown JA, Jenkins RB, Jensen RH. Comparative genomic hybridization, allelic imbalance, and fluorescence in situ hybridization on chromosome 8 in prostate cancer. Genes Chromosomes Cancer 1994;11:153–62.
- [22] El Gedaily A, Bubendorf L, Willi N, Fu W, Richter J, Moch H, Mihatsch MJ, Sauter G, Gasser TC. Discovery of new DNA amplification loci in prostate cancer by comparative genomic hybridization. Prostate 2001;46:184–90.
- [23] Speicher MR, du Manoir S, Schrock E, Holtgreve-Grez H, Schoell B, Lengauer C, Cremer T, Ried T. Molecular cytogenetic analysis of formalin-fixed, paraffin-embedded solid tumors by comparative genomic hybridization after universal DNA-amplification. Hum Genet 1993;2:1907–14.
- [24] Wiltshire RN, Duray P, Bittner ML, Visakorpi T, Meltzer PS, Tuthill RJ, Liotta LA, Trent JM. Direct visualization of the clonal progression of primary cutaneous melanoma: application of tissue microdissection and comparative genomic hybridization. Cancer Res 1995;55:3954–7.
- [25] Kuukasjarvi T, Tanner M, Pennanen S, Karhu R, Visakorpi T, Isola J. Optimizing DOP-PCR for universal amplification of small DNA samples in comparative genomic hybridization. Genes Chromosomes Cancer 1997;18:94–101.
- [26] Aubele M, Mattis A, Zitzelsberger H, Walch A, Kremer M, Welzl G, Hofler H, Werner M. Extensive ductal carcinoma in situ with small foci of invasive ductal carcinoma: evidence of genetic resemblance by CGH. Int J Cancer 2000;85:82–6.
- [27] Aubele M, Mattis A, Zitzelsberger H, Walch A, Kremer M, Hutzler P, Hofler H, Werner M. Intratumoral heterogeneity in breast carcinoma revealed by laser-microdissection and comparative genomic hybridization. Cancer Genet Cytogenet 1999;110:94–102.
- [28] Aubele M, Zitzelsberger H, Schenck U, Walch A, Hofler H, Werner M. Distinct cytogenetic alterations in squamous intraepithelial lesions of the cervix revealed by laser-assisted microdissection and comparative genomic hybridization. Cancer 1998;84:375–9.
- [29] Kim SH, Godfrey T, Jensen RH. Whole genome amplification and molecular genetic analysis of DNA from paraffin-embedded prostate adenocarcinoma tumor tissue. J Urol 1999;162:1512–8.
- [30] Kim S-H, Kim MS, Jensen RH. Genetic alterations in microdissected prostate cancer cells by comparative genomic hybridization. Prostate Cancer Prostatic Dis 2000;3:110–4.
- [31] Verhagen PC, Zhu XL. Rohr LR, Cannon-Albright LA, Tavtigian SV, Skolnick MH, Brothman AR. Microdissection, DOP-PCR, and comparative genomic hybridization of paraffin-embedded familial prostate cancers. Cancer Genet Cytogenet 2000;122:43–8.
- [32] Prasad MA, Trybus TM, Wojno KJ, Macoska JA. Homozygous and frequent deletion of proximal 8p sequences in human prostate cancers: identification of a potential tumor suppressor gene site. Genes Chromosomes Cancer 1998;23:255–62.
- [33] Wang JC, Radford DM, Holt MS, Helms C, Goate A, Brandt W, Parik M, Phillips NJ, DeSchryver K, Schuh ME, Fair KL, Ritter JH, Marshall P, Donis-Keller H. Sequence-ready contig for the 1.4-cM ductal carcinoma in situ loss of heterozygosity region on chromosome 8p22-p23. Genomics 1999;60:1–11.
- [34] Haggman MJ, Wojno KJ, Pearsall CP, Macoska JA. Allelic loss of 8p sequences in prostatic intraepithelial neoplasia and carcinoma. Urology 1997;50:643–7.
- [35] Perinchery G, Bukurov N, Nakajima K, Chang J, Hooda M, Oh BR, Dahiya R. Loss of two new loci on chromosome 8 (8p23 and 8q12-13) in human prostate cancer. Int J Oncol 1999;14:495–500.
- [36] Macoska JA, Beheshti B, Rhim JS, Hukku B, Lehr J, Pienta KJ, Squire JA. Genetic characterization of immortalized human prostate

epithelial cell cultures. Evidence for structural rearrangements of chromosome 8 and i(8q) chromosome formation in primary tumorderived cells. Cancer Genet Cytogenet 2000;120:50–7.

- [37] Virgin JB, Hurley PM, Nahhas FA, Bebchuk KG, Mohamed AN, Sakr WA, Bright RK, Cher ML. Isochromosome 8q formation is associated with 8p loss of heterozygosity in a prostate cancer cell line. Prostate 1999;41:49–57.
- [38] Alers JC, Krijtenburg PJ, Rosenberg C, Hop WC, Verkerk AM, Schroder FH, van der Kwast TH, Bosman FT, van Dekken H. Interphase cytogenetics of prostatic tumor progression: specific chromosomal abnormalities are involved in metastasis to the bone. Lab Invest 1997;77:437–48.
- [39] Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. Nature 1998;396:643–9.
- [40] Cahill D, Lengauer C, Yu J, Riggins G, Willson J, Markowitz S, Kinzler K, Vogelstein B. Mutations of mitotic checkpoint genes in human cancers. Nature 1998;392:300–3.
- [41] Egawa S, Uchida T, Suyama K, Wang C, Ohori M, Irie S, Iwamura M, Koshiba K. Genomic instability of microsatellite repeats in prostate cancer: relationship to clinicopathological variables. Cancer Res 1995;55:2418–21.
- [42] Dahiya R, Lee C, McCarville J, Hu W, Kaur G, Deng G. High frequency of genetic instability of microsatellites in human prostatic adenocarcinoma. Int J Cancer 1997;72:762–7.
- [43] Pihan GA, Doxsey SJ. The mitotic machinery as a source of genetic instability in cancer. Semin Cancer Biol 1999;9:289–302.
- [44] Pihan GA, Purohit A, Wallace J, Malhotra R, Liotta L, Doxsey SJ.

Centrosome defects can account for cellular and genetic changes that characterize prostate cancer progression. Cancer Res 2001;61: 2212–9.

- [45] Beheshti B, Park PC, Sweet JM, Trachtenberg J, Jewett MAS, Squire JA. Evidence of chromosomal instability in prostate cancer determined by spectral karyotyping (SKY) and interphase FISH analysis. Neoplasia 2001;3:62–9.
- [46] Olumi AF, Grossfeld GD, Hayward SW, Carroll PR, Tlsty TD, Cunha GR. Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. Cancer Res 1999;59:5002–11.
- [47] Hayward SW, Rosen MA, Cunha GR. Stromal-epithelial interactions in the normal and neoplastic prostate. Br J Urol 1997;79(Suppl 2):S18–S26.
- [48] Suzuki H, Freije D, Nusskern DR, Okami K, Cairns P, Sidransky D, Isaacs WB, Bova GS. Interfocal heterogeneity of PTEN/MMAC1 gene alterations in multiple metastatic prostate cancer tissues. Cancer Res 1998;58:204–9.
- [49] Hugel A, Wernert N. Loss of heterozygosity (LOH), malignancy grade and clonality in microdissected prostate cancer. Br J Cancer 1999;79:551–7.
- [50] Saric T, Brkanac Z, Troyer DA, Padalecki SS, Sarosdy M, Williams K, Abadesco L, Leach RJ, O'Connell P. Genetic pattern of prostate cancer progression. Int J Cancer 1999;81:219–24.
- [51] Rohrbach H, Haas CJ, Baretton GB, Hirschmann A, Diebold J, Behrendt RP, Lohrs U. Microsatellite instability and loss of heterozygosity in prostatic carcinomas: comparison of primary tumors, and of corresponding recurrences after androgen-deprivation therapy and lymphnode metastases. Prostate 1999;40:20–7.