Urol Res (1999) 27:3–8
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ORIGINAL PAPER

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Characterization of chromosome 8 aberrations in the prostate cancer cell line LNCaP-FGC and sublines

Received: 2 May 1997 / Accepted: 27 October 1997

Abstract In two androgen-dependent (FGC and P70) and two androgen-independent (LNO and R) sublines of the prostate cancer model LNCaP numerical and structural aberrations of chromosome 8 were investigated in detail. The techniques used were whole chromosome paint (WCP) and fluorescence in situ hybridization (FISH) with three cosmid probes mapping to different parts of the p-arm (D8S7 (8p23.3), LPL (8p22) and PLAT (8p11.1)). By WCP all four cell lines showed four copies of chromosome 8 in most cells. However, FISH demonstrated that in all sublines deletions in the 8p region were present. The majority of both FGC and P70 had two copies of cosmids D8S7 and LPL. The cosmid PLAT showed a broader distribution (1–4 copies), especially in P70. Compared with FGC and P70, both LNO and R showed a larger number of copies (3 or 4) of all three cosmid loci. It is discussed that this difference is probably the result of nondisjunction as a reaction to loss of other sequences on 8p, possibly the tumor suppressor gene (TSG) mapping to 8p21. The fact that both sublines LNO and R are androgen-independent raises the possibility of a link between TSG loss on 8p and androgen independence.

Key words Prostatic carcinoma · Chromosome 8p · Androgen independence · Fluorescence in situ hybridization (FISH) · Tumor suppressor gene (TSG)

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Introduction

Recently we demonstrated that in patients with prostate carcinoma (PC), abnormalities of the number of copies of chromosome 8 in the tumor are correlated with an advanced tumor stage [9]. In a previous cytogenetic study of sublines of the prostatic carcinoma cell line LNCaP [8], we observed that the two androgen-independent sublines R and LNO both had breakpoints in chromosome 8p, which resulted in various marker chromosomes. In that study such breakpoints and markers were not observed in the other, androgen-dependent, sublines. Since it is well known that advanced tumors are often nonresponsive to hormonal therapy, it was concluded that these rearrangements would possibly have a relation with progression to androgen independence in these cell lines. Recently the interest in the 8p region has greatly increased, thanks to the postulated location in that region of one or more tumor suppressor genes (TSGs), which may be also important in PC progression [1, 2, 12, 16]. The present study adds new information to the cytogenetic data of the LNCaP PC model. A high passage of the parental FGC line [11] has been characterized separately and additional information about the chromosome 8 rearrangements in the other four sublines is reported. These results were obtained by the molecular cytogenetic techniques whole chromosome paint (WCP) and fluorescence in situ hybridization (FISH) with cosmid probes. The different rearrangements found in the sublines are discussed in relation to their biological behavior.

Materials and methods

PC cell lines, culture and androgen responsiveness

All PC cell lines used in this study, i.e., FGC at passage 20 (FGC), FGC at passage 70 (P70), LNO and LNCaP-r (R), were sublines established from the original LNCaP cell line (Fig. 1) [6, 11, 15]. FGC and LNO were kindly provided by Dr J. Horoszewicz

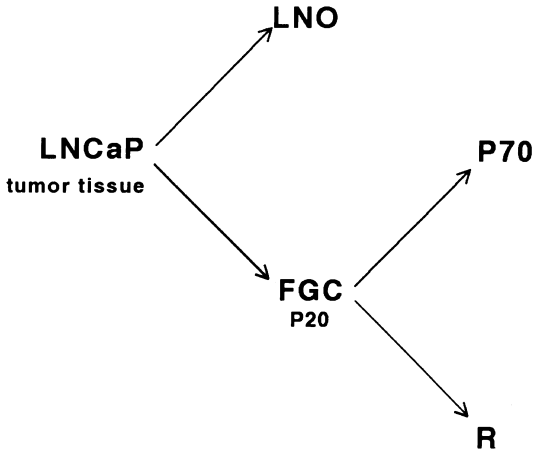


Fig. 1 Schematic presentation of the lineage of sublines from LNCaP

(Roswell Park Memorial Institute, Buffalo, USA). It is important to note here that the cell line we call FGC is identical to the cell line LNCaP-FGC that is available through the ATCC. The R line [5] was a gift from Dr M. Hasenson (Kardinska Institute, Sweden). The P70 subline is a higher passage culture which originated from FGC after prolonged (1 year) in vitro culture, replating once weekly [11]. FGC, P70 and R were routinely cultured in RPMI medium, supplemented with 10% fetal calf serum (FCS), glutamin and antibiotics. For the culture of LNO, regular FCS was replaced by 5% steroid-depleted FCS. FGC is androgen-dependent, which means that the cells will not grow in androgen-depleted medium [15]. FGC cells respond with dose-dependent stimulation of proliferation to the addition of androgens. P70 has lost the complete dependence of androgens and thus also grows in their absence, but it still responds to the addition of androgens with increased proliferation [11], i.e., these cells are androgen-independent, but sensitive. LNO and R are both androgen-independent and insensitive: both grow well on steroid-depleted serum and will not grow faster when androgens are added [5, 15]. However, these two sublines are different with respect to their lineage from the parent cell line (see Fig. 1) and with respect to their karyotype [8].

Karyotypes

The karyotypes of FGC, LNO and R have been described in detail previously [8]. For the present study the karyotype of these cell lines was checked by R-banding of metaphases as described previously. P70 arose later and was characterized in the same manner. All four cell lines were near tetraploid with median chromosome numbers of, respectively, 86 (FGC), 80 (LNO), 87 (R), and 91 (P70). All showed the original LNCaP marker chromosomes, involving chromosomes 1, 2, 3, 4, 6, 10, 13, 15, and 16, as well as each of their subline specific additional marker chromosomes. P70 showed only one specific marker, namely the same 1p+ marker as its parent cell line FGC (in all karyotyped cells).

FISH procedure

The cosmid probes for three unique chromosome 8p loci (D8S7, 11E1/8p23.3, LPL, 114C11/8p22 and PLAT, 105H8/8p11.1) were kindly provided by Dr S. Wood (Vancouver, Canada) [18]. The order of the cosmids from telomere to centromere is: D8S7-LPL-PLAT. The chromosome 8 centromere probe was D8Z2 [3]. The chromosome 8 paint was from Cambio (UK). Hybridization and detection of cosmids and paint were performed essentially as described previously for chromosome-specific DNA probes [9], with some modifications. For cosmid hybridization 100 ng biotinylated cosmid DNA and 10 μg Cot-1 DNA (Gibco BRL) per slide were added to the hybridization mixture in 50% formamide. This mixture was denatured at 72 °C for 4 min, followed by preannealing at 37 °C for 2 h. Hybridization to metaphases on slides occurred during overnight incubation at 37 °C in a moist chamber. For the chromosome 8 paint 200 ng biotinylated chromosome 8 DNA and 15 μg Cot-1 DNA were used per slide. The same protocol as for cosmid DNA was followed. In both cases detection was by FITC-avidin. For double-color FISH analysis with both the centromere probe and either D8S7 or LPL, the cosmid was digoxygenated (Boehringer). Detection was by Texas Red-anti-dig. Combination of the centromere probe with PLAT was not possible, due to the near-centromeric localization of PLAT. DNA was counterstained with propidium iodide. For identification of chromosomes DAPI (4′,6-diamidino-2-phenylindol) stain (0.8 μg/ml) was also applied to the same slides.

FISH efficiency, use of WCP and definition of deletion

Between 50 and 100 metaphases of each cell line were scored per hybridization experiment. Hybridization efficiency for the three cosmid probes was evaluated using normal lymphocytes and was about 80% for all three, as described by Matsuyama et al. [13]. We used WCP instead of the centromere probe to determine the ploidy of chromosome 8 for the following reasons. (1) Both techniques gave comparable results; as an example: in LNO the ploidy distribution of 8 by WCP vs by centromere probe was 4% vs 6% for two copies, 17% vs 18% for three copies, 74% vs 72% for four copies and 5% vs 4% for more than four copies). (2) By employing WCP, the ploidy as well as structural rearrangements could be evaluated in the same experiment. (3) Because we evaluated metaphases, and with WCP a bigger target is colored, detection was easier. The partially tetraploid (63% of the cells) human B lymphoblastoid cell line JY [14], that has no aberrations in chromosome 8 (checked by karyotype analysis) was used as a control for hybridization of the cosmids to tetraploid cells. In all tetraploid metaphases of JY three or four signals were detected for D8S7. The percentage of cells with three signals was 22 ± 3.6%. The cut-off level of deletion was set to 30% (mean + 2 × SD). This cut-off was also applied to LPL and PLAT, because these behaved similarly. So in cosmid experiments with four signals in less than 70% of the tumor metaphases, at least one copy of the probe was considered deleted. Because no metaphases with less than three signals were seen in the control cell line, in tumor metaphases with zero, one or two signals, respectively, all, three or two copies were considered deleted.

Results

Aberrations of chromosome 8 by paint analysis

FGC

Paint analysis of metaphases of FGC demonstrated that the main population of cells showed four copies of chromosome 8 (82%). Ten percent of the cells had more than four, 8% three, and a minor population two copies of chromosome 8 (Table 1). Marker chromosomes with microscopically visible 8p and 8q aberrations were mainly detected in cells with, in total, four copies of chromosome 8 (Table 2). These were mostly resulting from translocations of other material to 8p or 8q (Fig. 2A), concurrent with partial deletion of 8p or 8q DNA. Also several independent chromosome fragments entirely consisting of chromosome 8 DNA were detected (3.2%).

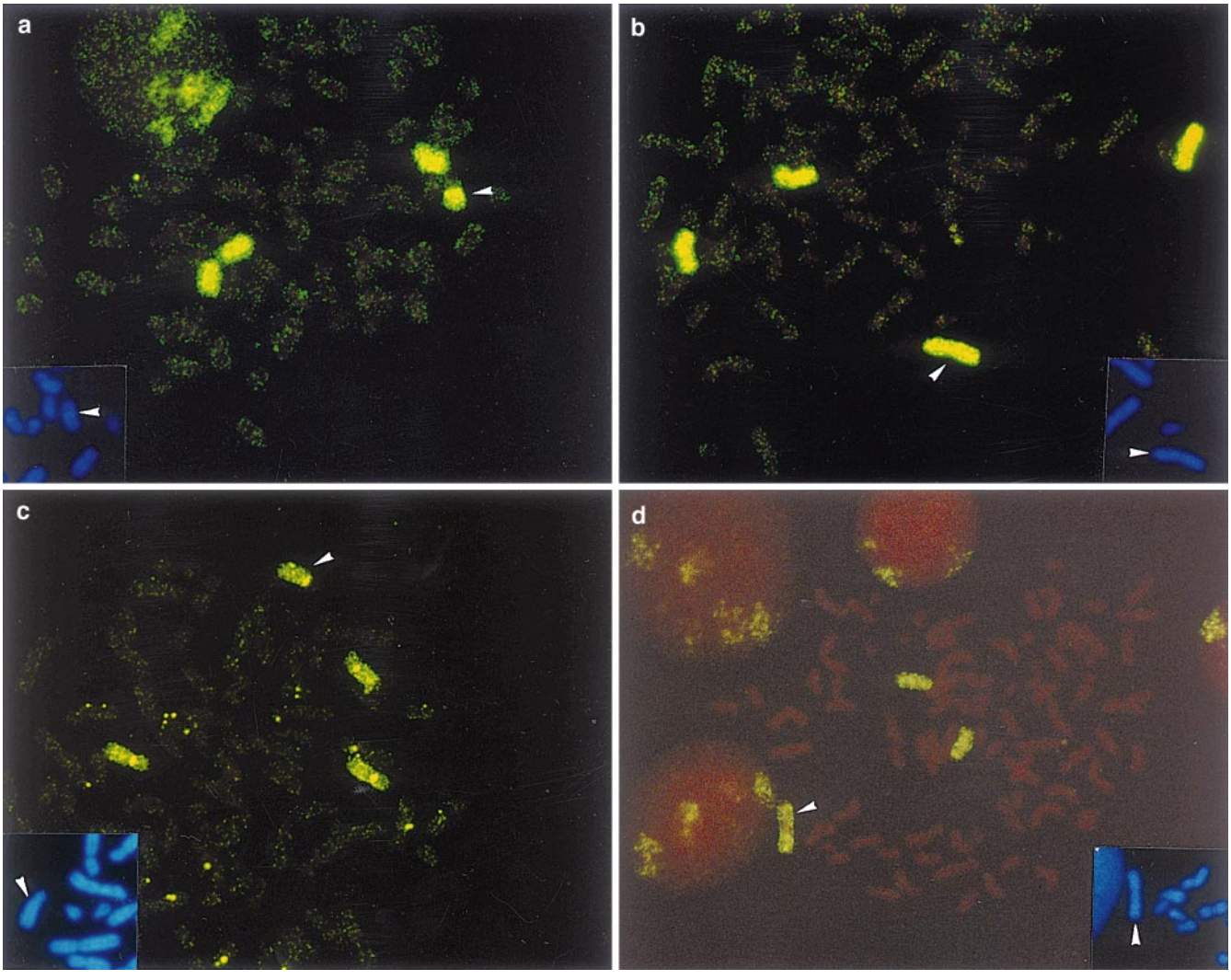
Table 1 Results of whole chromosome paint (WCP) and cosmid hybridization studies; percentages of metaphases with a positive hybridization signal

No. of Copies	FGC				P70				LNO				R			
	WCP	D8S7	LPL	PLAT	WCP	D8S7	LPL	PLAT	WCP	D8S7	LPL	PLAT	WCP	D8S7	LPL	PLAT
1	0	23	12	28	0	7	19	4	0	3	7	7	0	0	8	0
2	2	68	69	39	3	47	52	26	4	42	36	21	2	39	20	22
3	8	8	20	33	7	37	27	52	17	52	46	36	24	32	28	27
4	82	3	0	0	84	9	2	17	74	3	11	25	59	19	44	40
>4	10	0	0	0	6	0	0	0	5	0	0	12	15	0	0	11

Table 2 Percentages of chromosome 8 aberrations as detected by WCP

	FGC	P70	LNO	R
p-arm	8.0	4.4	4.0	9.9
q-arm	6.4	1.5	1.0	7.1
Unidentified	3.2	4.4	0.0	0.8
All	17.6	10.3	5.0	17.8

Fig. 2A–D Detection of chromosome paint DNA on all metaphases shown was by FITC avidin, propidium iodide counterstain. Verification of chromosomes was done with DAPI stain (see lower right-hand corner part). Magnification: ×312.5. Chromosome 8 paints on metaphases of: (**A**) FGC. Partial metaphase, showing three normal chromosomes 8 and one marker (*arrow*), resulting from translocation of material of another chromosome to 8p. (**B**) P70. Three normal chromosomes 8 and a large marker chromosome (*arrow*), probably an i(8q). (**C**) LNO. Three normal chromosomes 8 and a marker chromosome with the whole p-arm deleted (*arrow*). (**D**) R. Two normal chromosomes 8 and a large marker chromosome, entirely consisting of chromosome 8 material (*arrow*) are shown



P70

Paint analysis of P70 showed the close relationship to FGC in the nearly equal distribution of chromosome 8 copies (Table 1). However, 8p- and q-arm aberrations occurred less frequently in P70 than in FGC (Table 2). The most prominent aberration was an A-sized marker, consisting only of chromosome 8 DNA (in 4.4% of the cells). With DAPI stain, this marker showed a banding pattern that could fit an i(8q) (Fig. 2B). In P70 metaphases the highest percentage of unidentified chromosome 8 material was found (4.4%). Both independent chromosome fragments, like in FGC, and translocated material to other chromosomes were seen.

LNO

Paint analysis of LNO showed 74% of the cells with four copies of chromosome 8, 17% cells with three copies, and some cells with two or more than four copies (Table 1). Occasionally q-arm deletions were seen restricted to cells with three copies of 8. However, the majority of aberrations found were p-arm deletions of different sizes in cells with four copies of 8 (Table 2 and Fig. 2C). The 8p- marker that was described earlier (del(8)(p21-pter)) was still present, but its frequency had declined to 6%. Unidentified fragments were not detected.

R

In R substantial proportions of three (24%) and more than four (15%) copies of 8 were detected by WCP (Table 1). P-or q-arm abnormalities occurred in cells with three, four, as well as more than four copies of chromosome 8 (Table 2). However, the 8p+ marker that was described previously (8qter-8p23::8q13-8qter)) occurred far less frequently (3.3%). Probably this marker was further modified, resulting in other chromosome 8 markers as demonstrated in Figure 2D. Independent fragments consisting of chromosome 8 material were seen only occasionally (0.8%). FGC and R showed the highest overall percentage of metaphases with chromosome 8 aberrations (17.6% and 17.8% respectively).

Aberrations of 8p detected with cosmids in relation to the number of copies of chromosome 8

In all four cell lines investigated and for all three cosmids, the percentages of cells with four copies of cosmids were invariably below the cut-off value of 70% that marks loss of one copy. Consequently the frequency distributions presented in Table 1 can be regarded also as distribution patterns for loss of cosmid sequences. PLAT was present on 100% of the 8p− marker chromosomes, found in any subline. Paint analysis of FGC showed predominantly four copies of 8, but by cosmid analysis mainly two copies of D8S7 and LPL were detected. However, populations with one or three copies of these two cosmids were also seen with moderate frequency. PLAT showed fewer cells with two copies, but more cells with one and three copies. D8S7 showed four copies in some cells, but LPL and PLAT did not. In P70, as in FGC, with WCP most cells had shown four copies of chromosome 8. In concordance with FGC, P70 mainly showed two copies for both D8S7 and LPL. However, the populations of cells with three copies of both these cosmids had increased in P70. PLAT showed mainly three copies, and a significant population with four copies, although still more than a quarter of the cells maintained two copies. Also in contrast to the results for FGC was the observation that in P70, LPL showed more cells with one copy than D8S7 and PLAT. Double-color FISH analysis with D8Z2 for the centromere and the cosmids D8S7 or LPL (PLAT could not be evaluated in this way due to its position near the centromere) demonstrated that in cells with three copies of 8, D8S7 and LPL were present in only two copies. This indicated that in these cells one of the aberrant chromosomes was lost (not shown). Although paint analysis of LNO showed largely the same distribution as FGC, the cosmid analysis showed different results. Two as well as three copies of D8S7 and LPL were most frequent in this subline. Moreover, PLAT demonstrated a completely different distribution, showing a clear shift to more copies. Double-color FISH analysis showed that in cells with three copies of 8, like in P70, one of the aberrant chromosomes was lost. In R, of which 15% of the cells had more than four copies of chromosome 8, only PLAT was present in more than four copies. However, in relation to the other sublines, all three cosmids demonstrated a clear shift towards more copies, whereby LPL and PLAT both showed a majority of cells with four copies. Double-color FISH analysis showed the same result as for P70 and LNO.

Discussion

Painting analysis showed that in all four LNCaP sublines the majority of the cells had four, apparently normal, copies of chromosome 8. FGC and P70 showed only minor populations with aberrations from tetraploidy, never exceeding 10% of the cells. However, LNO, and especially R, both androgen-independent and both descended from the parental cell line at an early stage of in vitro culture, displayed larger cell populations with three (LNO, R) or even more than four copies (R). The findings described here demonstrate that tumor cell lines growing in vitro are dynamic entities, just like tumors growing in vivo. Inherent chromosomal instability allows the cells to constantly generate new chromosomal aberrations. These will either quickly be lost again or be retained, dependent on the benefit the cell gains from them, while reacting on genetic or environmental pres-

sure. The presence of the 8p aberrations found previously in LNO and R [8] were confirmed by WCP in the present study. Also new, not previously described aberrations of chromosome 8, involving both 8p and 8q sequences, were discovered in these cell lines. Such aberrations were also demonstrated for the first time by WCP in the sublines FGC and P70.

The most surprising finding of the present study was that, despite the apparent presence of four normal copies of chromosome 8 in most cells of the investigated cell lines, only few cells seemed to have retained all 8p sequences on all chromosomes. The deletion patterns found reflect the lineage of the different sublines as depicted in Fig. 1. Molecular genetic analysis in the 8p22-8cen region showed the close relationship between FGC and P70 through their identical microsatellite repeat patterns (pers. comm. W. Dinjens, Department of Pathology, EUR). It is conceivable that in the original diploid cell line (LNCaP in Fig. 1) [4, 6] only one chromosome 8 had one or more deletions and that these mapped only distal to 8p22. Combined with the results of the FISH analysis of FGC and P70 for PLAT that maps proximal, and D8S7 and LPL that both map distal to 8p22, it can be assumed that after tetraploidization several new structural aberrations were generated in the normal as well as in the pre-existing aberrant chromosomes. These events finally resulted in the deletion patterns observed for both the low passage cells (FGC) and the high passage cells (P70).

The deletion patterns observed for the sublines LNO and R can only be explained by the occurrence of several nondisjunction events in addition to the tetraploidization and chromosome breakage, as described above for FGC and P70. Interestingly, analysis of CA repeats on 8p proximal to band p22 in both LNO and R showed differences in several markers when compared to FGC (pers. comm. W. Dinjens). However, these data are preliminary and they need to be reconfirmed before definite conclusions can be drawn. If the deletions found by cosmid analysis were continuous between the regions investigated, two chromosomes with microscopically visible 8p deletions were expected in most cells of FGC, P70 and LNO, and one in R. However, by paint analysis maximally 10% of the metaphases with such aberrations could be identified. A more likely explanation for these findings would be that the deletions were mostly discontinuous and relatively small, i.e., spanning less than one band. Using comparative genome hybridization, large deletions on 8p, sometimes spanning most of the p-arm, have been detected in a number of PC specimens [2, 7, 17]. However, with LOH mapping interspersion of deleted and retained regions on chromosome 8 has also been observed [12, 16], so it is conceivable that this is the case in the LNCaP sublines investigated.

Matsyama et al. [13] found that in poorly differentiated clinical PC both D8S7 and LPL were lost. This is in accordance with our results, since the LNCaP cell line was derived from a poorly differentiated lymph node metastasis [6]. However, in more differentiated clinical tumors, it was found that D8S7 was mostly retained [13]. This result is suggestive of two TSGs that might be inactivated at different stages in the progression of PC. In other studies allelic loss on 8p generally was associated with higher tumor grade, and the deletion patterns found were suggestive of one or two TSG [2, 12, 16]. Discordant results in two separate LOH studies, one narrowing down the locus for a putative TSG to 17cM in the 8p21 region [16], and the other reporting on a patient with a homozygous deletion in the 8p22 region [1], can be brought together by the concept of two TSGs on 8p, as was also suggested for colorectal cancer [19]. Following this concept, it is conceivable that in FGC and P70 only the 8p22 TSG was inactivated, and in LNO and R both TSGs. We postulate that the shift towards more copies of certain 8p sequences in LNO and also in R is probably the result of nondisjunction as a reaction to partial loss of other sequences on 8p [2], possibly the TSG mapping to 8p21. (This mechanism may even be typical for more advanced tumors [10].)

As LNO is a very early descendant from the parental cell line, its differences from FGC and P70 could have been already present before in vitro culture. However, in the case of R, which originated from FGC long after its establishment as an in vitro cell line [5], the significant chromosomal rearrangements must have taken place during in vitro culture. The present results once more emphasize the many ways in which tumor models can be employed. Moreover, if other variant cell lines of FGC can be generated by culture without androgens and if they show similar rearrangements, the link between the androgen-independent and insensitive status and the phenomenon of loss of sequences on 8p in the LNCaP human prostate tumor cell line can be made more firmly. Then, to confirm the significance of this finding, patient tissues at various clinical stages should be investigated for these same rearrangements.

Acknowledgement This study was supported by the Dutch Cancer Society.

References

1. Bova GS, Carter BS, Bussemakers MJG, Emi M, Fujiwara Y, Kyprianou N, Jacobs SC, Robinson JC, Epstein JI, Walsh PC, Isaacs WB (1993) Homozygous deletion and frequent allelic loss of chromosome 8p22 loci in human prostate cancer. Cancer Res 53:3869
2. Cher ML, MacGrogan D, Bookstein R, Brown JA, Jenkins RB, Jensen RH (1994) Comparative genomic hybridization, allelic imbalance, and fluorescence in situ hybridization on chromosome 8 in prostate cancer. Genes Chromos Cancer 11:153
3. Donlon T, Wyman AR, Mulholland J, Barker D, Bruns G, Latt S, Botstein D (1986) α satellite-like sequences at the centromere of chromosome 8. Am J Hum Genet 39:A196
4. Gibas Z, Becher R, Kawinski E, Horoszewicz J, Sandberg AA (1984) A high-resolution study of chromosome changes in a human prostatic carcinoma cell line (LNCaP). Cancer Genet Cytogenet 11:399

5. Hasenson M, Hartley-Asp B, Kihlfors C, Lundin A, Gustafsson J, Pousette A (1985) Effect of hormones on growth and ATP content of a human prostatic carcinoma cell line, LNCaP-r. Prostate 7:183
6. Horoszewicz JS, Leong SS, Kawinski E, Karr JP, Rosenthal H, Ming Chu T, Mirand EA, Murphy GP (1983) LNCaP model of human prostatic carcinoma. Cancer Res 43:1809
7. Joos S, Bergerheim USR, Pan Y, Matsuyama H, Bentz M, Manoir S du, Lichter P (1995) Mapping of chromosomal gains and losses in prostate cancer by comparative genomic hybridization. Genes Chromos Cancer 14:267
8. König JJ, Kamst E, Hagemeijer A, Romijn JC, Horoszewicz J, Schröder FH (1989) Cytogenetic characterization of several androgen responsive and unresponsive sublines of the human prostatic carcinoma cell line LNCaP. Urol Res 17:79
9. König JJ, Teubel W, Dongen JW van, Romijn JC, Hagemeijer A, Schröder FH (1994) Loss and gain of chromosomes 1, 18 and Y in prostate cancer. Prostate 25:281
10. König JJ, Teubel W, Romijn JC, Schröder FH, Hagemeijer A (1996) Gain and loss of chromosomes 1, 7, 8, 10, 18 and Y in 46 prostate cancers. Hum Pathol 27:720
11. Langeler EG, Uffelen CJC van, Blankenstein MA, Steenbrugge GJ van, Mulder E (1993) Effect of culture conditions on androgen sensitivity of the human prostatic cancer cell line LNCaP. Prostate 23:213
12. MacGrogan D, Levy A, Bostwick D, Wagner M, Wells D, Bookstein R (1994) Loss of chromosome arm 8p loci in prostate cancer: mapping by quantitative allelic imbalance. Genes Chromos Cancer 10:151
13. Matsuyama H, Pan Y, Skoog L, Tribukait B, Naito K, Ekman P, Lichter P, Bergerheim USR (1994) Deletion mapping of chromosome 8p in prostate cancer by fluorescence in situ hybridization. Oncogene 9:3071
14. Reboul A, Arvisux J, Wright JF, Colomb MG (1991) Proteolytic fragmentation of tetanus toxin by subcellular fractions of JY, a B lymphoblastoid cell line. Biochem J 277:47
15. Steenbrugge GJ van, Uffelen CJC van, Bolt J, Schröder FH (1991) The human prostatic carcinoma cell line LNCaP and its derived sublines: an in vitro model for the study of androgen sensitivity. J Ster Biochem 40:207
16. Trapman J, Sleddens HFBM, Weiden MM van der, Dinjens WNM, König JJ, Schroder FH, Faber PW, Bosman FT (1994) Loss of heterozygosity of chromosome 8 microsatellite loci implicates a candidate tumor suppressor gene between the loci D8S87 and D8S133 in human prostate cancer. Cancer Res 54:6061
17. Visakorpi T, Kallioniemi AH, Syvanen AC, Hyytinen ER, Karhu R, Tammela T, Isola JJ, Kallioniemi OP (1995) Genetic changes in primary and recurrent prostate cancer by comparative genomic hybridization. Cancer Res 55:342
18. Wood S, Schertzer M, Drabkin H, Patterson D, Longmire JL, Deaven LL (1992) Characterization of a human chromosome 8 cosmid library constructed from flow-sorted chromosomes. Cytogenet Cell Genet 59:243
19. Yaremko ML, Wasylyshyn ML, Paulus KL, Micheiassi F, Westbrook CA (1994) Deletion mapping reveals two regions of chromosome 8 allele loss in colorectal carcinoma. Genes Chromos Cancer 10:1