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Identification of a high frequency of chromosomal rearrangements in the centromeric regions of prostate cancer patients

BALACHANDAR V.†, LAKSHMAN KUMAR B., SASIKALA K.,
MANIKANTAN P., SANGEETHA R., MOHANA DEVI S.

(*Division of Human Genetics, Department of Zoology, Bharathiar University, Coimbatore-641046, Tamilnadu, India*)

†E-mail: geneticbala@yahoo.co.in

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Abstract: The aim of the present investigation was to study the major chromosomal aberrations (CA) like deletion, translocation, inversion and mosaic in prostate cancer patients of Tamilnadu, Southern India. Totally 45 blood samples were collected from various hospitals in Tamilnadu, Southern India. Equal numbers of normal healthy subjects were chosen after signing a consent form. Volunteers provided blood samples (5 ml) to establish leukocyte cultures. Cytogenetic studies were performed by using Giemsa-banding technique and finally the results were ensured by spectral karyotyping (SKY) technique. In the present investigation, major CA like deletion, translocation, inversion and mosaic were identified in experimental subjects. Results showed frequent CA in chromosomes 1, 3, 5, 6, 7, 9, 13, 16, 18 and X. In comparison with experimental subjects, the control subjects exhibited very low levels of major CA ($P<0.05$). In the present study, the high frequency of centromeric rearrangements indicates a potential role for mitotic irregularities associated with the centromere in prostate cancer tumorigenesis. Identification of chromosome alterations may be helpful in understanding the molecular basis of the disease in better manner.

Key words: Prostate cancer, Chromosomal aberrations (CA), Giemsa-banding, Spectral karyotyping (SKY)
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INTRODUCTION

Prostate cancer is one of the leading cancer types and is the second most common cause of cancer mortality in men in North America (Haas and Sakr, 1997). An influence of diet and environment on prostate cancer risk is suggested by studies in Asian countries and Japanese men who migrated to the US (Cook *et al*., 1999; Whittemore *et al*., 1995). In India, the annual mortality in 2000 was 0.7 million, and the annual estimate of cancer for the year 2001 was 0.98 million (Greenlee *et al*., 2001). It is relatively rare for prostate cancer to be diagnosed in men below 50 years of age, but above this age, the incidence and mortality rates increase exponentially (Haas and Sakr, 1997). The age-specific incidence curve for prostate cancer has a steeper slope than for any other cancer and with the present trend toward an aging population, prostate cancer is a major public health concern (Ross *et al*., 1979). Nevertheless, the etiology of prostate cancer has not been clearly elucidated, nor is the molecular mechanisms of the disease development and progression well characterized. The most generally accepted model of carcinogenesis postulates that cancer develops through the accumulations of genetic alterations that allow the cells to escape normal growth-regulatory mechanisms (Nowell, 1986).

There has been an ever-growing literature on chromosomal aberrations (CA) in prostate cancer over the past few years. Initial studies suggested that ~75% of prostate cancer tumors had normal male karyotypes. Several candidate chromosomes such as 1, 7, 8, 10, 17, and X are known to play a vital role in the development of prostate cancer (Gibbs *et al*., 1999; Zucchi *et al*., 1999). The frequency of chromosome instability in peripheral blood lymphocytes is relevant biomarker for cancer risk in humans, reflecting early biological effects of genotoxic carcinogens and indi-

vidual cancer susceptibility (Bonassi *et al*., 2000; Hagmar *et al*., 1998). An increased frequency of CA in circulating lymphocytes is generally considered indicative of increased cancer risk for those exposed to DNA damaging agents (Bonassi *et al*., 1995). Although extensive work has been carried out on prostate cancer (Steiner *et al*., 2002; Verhagen *et al*., 2002; Wolter *et al*., 2002), still there are no concrete reports available about the spontaneous background levels of CA in the peripheral blood lymphocytes of prostate cancer patients with respective to the age group. In the present study, the frequency of CA in peripheral blood lymphocytes has been studied in prostate cancer patients (40~80 years) of Tamilnadu and compared with normal healthy subjects of similar age groups to address this issue.

MATERIALS AND METHODS

Subject recruitment and sample collection

A total of 90 subjects aged 40~80 years old including 45 prostate cancer patients and 45 healthy controls, were recruited and subdivided into 4 age groups, namely group I (40~50 years), group II (51~60 years), group III (61~70 years) and group IV (71~80 years). Five millilitres of blood samples were collected from 45 prostate cancer patients (different age groups) in various hospitals of Tamilnadu, who did not undergo any treatment such as hormonal therapy, chemotherapy and radiation therapy. The prostate-specific antigen (PSA) level of collected blood samples was ≤10.0 mg/ml in 39 subjects (86.7%) and ≥10.0 mg/ml in 6 subjects (13.3%). Forty-five healthy controls were selected from the same area as that of the patients. All controls had PSA levels <4.0 mg/ml (90%) and 10% of them had PSA levels <2.0 mg/ml along with confirmation by digital rectal exam. Data on medical and family history of cancer, smoking habits, and occupational history were obtained through an interviewer-administered questionnaire as well as from review of the patient's hospital records of both control and cancer patients.

Chromosomal aberration assay

All chemical reagents were purchased from Sigma Chemicals (USA), except colcemid that was obtained from Gibco Laboratory (USA). Blood samples were set up to establish leukocyte cultures following standard procedures in our laboratory (Hoyos *et al*., 1996). Briefly, 0.5 ml blood was added to 4.5 ml RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mmol/L L-glutamine, 1% streptomycin-penicillin, 0.2 ml reagent grade phytohemagglutinin, and was incubated at 37 °C. After 50 h, cultures were treated with 0.1 g/ml colcemid to block cells in mitosis. Lymphocytes were harvested after 52 h by centrifuging cells to remove culture medium (800~1000 r/min), added with hypotonic solution (KCl, 0.075 mol/L) at 37 °C for 20 min to swell the cells, and treated twice with Carnoy's fixative (3:1 (v/v) ratio of methanol:acetic acid). Slides were carefully dried on a hot plate (56 °C, 2 min), and then stained using the Giemsa-banding (G-banding) technique (Goto *et al*., 1975). Microscope coordinates of all digitized G-banded preparations were recorded so that the metaphase cells analyzed by G-banding technique could be analyzed concurrently by spectral karyotyping (SKY) methods.

Spectral karyotyping (SKY)

The SKY™ kit probe cocktail from Applied Spectral Imaging (ASI, Carlsbad, CA, USA) was hybridized to metaphase spreads from each prostate cancer slides according to standard protocols (Garini *et al*., 1996; Schrock *et al*., 1996; Veldman *et al*., 1997) and as per the manufacturer's instructions (ASI, Carlsbad, CA, USA). After destaining the G-banded slides with methanol for 10 min, the slides were rehydrated in a descending ethyl alcohol series (100%, 90%, 70%), and fixed with 1% formaldehyde in 50 mmol/L $MgCl_2$/phosphate buffer solution for 10 min. The slides were then dehydrated using an ascending ethyl alcohol series and denatured for 30~45 s in 70% formamide/2× SSC at 75 °C. The SKY probe was denatured for 7 min at 75 °C, reannealed at 37 °C for 1 h, placed on the slide and covered with a glass coverslip. The coverslip was sealed with rubber cement and the slides placed in a damp container in a 37 °C incubator. After overnight hybridization, the post-hybridization washes were performed as per manufacturer's instructions (ASI, Carlsbad, CA, USA).

The metaphase images were captured using an SD 200 spectral bio-imaging system attached to a Zeiss microscope and stored on a SKY image-capture

workstation. The images were analyzed using the SKY View software version 1.2 which resolves individual fluorochrome spectra by Fourier spectroscopy and distinguishes the spectral signatures for each chromosome to provide a unique pseudocolour for each chromosome (classified image).

Statistical analysis

All statistical analyses were performed using software SPSS for Windows, version 13. To assess the differences between prostate cancer patients and controls, variables expressed by the mean±*SE* were analyzed using paired *t*-tests. $P<0.05$ was used as the criterion for significant difference between the groups.

RESULTS

Selected prostate cancer patients with CA are shown in Table 1. Results showed that CA has gradually increased in subjects with respect to their age (Fig.1). The mean±*SE* was 2.40±0.50 (group I), 2.60±0.21 (group II), 4.35±0.29 (group III), and 8.60±1.56 (group IV). In Table 1, the CA were frequently exhibited in chromosomes 1, 2, 5, 6, 7, 9, 12, 14, 16, 18, 22 and X. Interestingly, controls displayed very low level of major CA (Table 2). In the controls, the mean±*SE* was 0.80±0.58, 0.46±0.13, 0.95±0.18 and 2.40±0.67 in groups I, II, III and IV, respectively. Statistically significant results were obtained from paired *t*-test ($P<0.05$). From the above data, it can be inferred that significant elevation in CA was observed in prostate cancer patients with increase of age when compared with that of control age groups (Fig.2).

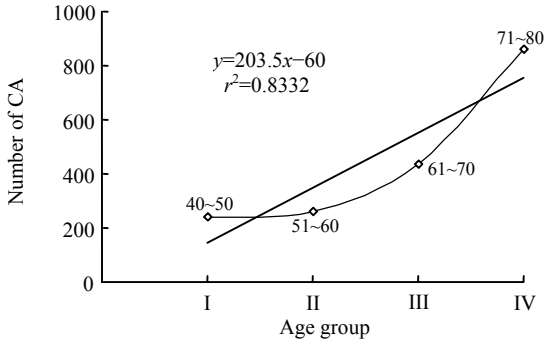

Fig.1 Correlation for the total number of chromosomal aberrations (CA) in 100 cells scored per subject prostate cancer. The CA recorded is calculated as per 100 subjects in each group

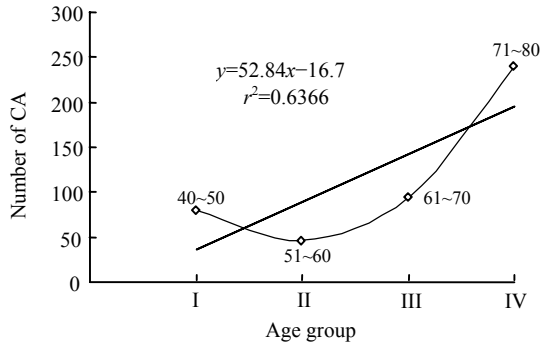

Fig.2 Correlation for the total number of chromosomal aberrations (CA) in 100 cells scored per control. The CA recorded is calculated as per 100 controls in each group

DISCUSSION

Cancer is a consequence of genetic or epigenetic alterations in a variety of genes that are fundamental to the process of growth, cell proliferation, differentiation and programmed cell death (Sandberg, 1991). Each alteration whether an initiating or a progression-associated event, may be mediated through gross chromosomal change and hence has the potential to be detected cytogenetically (Solomon *et al*., 1991). However, a low level of chromosomal instability is detectable in the peripheral blood lymphocytes of patients with skin, breast and bladder cancers and lymphomas (Barrios *et al*., 1990; Madhavi *et al*., 1990). Bonassi *et al*.(1995) reported a significant increase in the mortality ratio for all cancers in subjects who had shown increased levels of CA in their lymphocytes. Essentially, the data from both these studies when pooled indicated that the frequency of chromosome instability in peripheral blood lymphocytes is a relevant biomarker for cancer risks in humans, reflecting early biological effects of genotoxic carcinogens and individual cancer susceptibility (Bonassi *et al*., 2000; Hagmar *et al*., 1998).

Although previous studies have shown the presence of chromosome instability in prostate tumors (Verhagen *et al*., 2002; Wolter *et al*., 2002), this is the first molecular cytogenetic study to investigate the major CA like deletion, translocation, inversion and mosaics in the peripheral blood lymphocytes of previously untreated prostate cancer patients from Tamilnadu, Southern India among different age groups.

Table 1 Chromosomal abnormalities in prostate cancer patients identified by sequential G-banding and SKY techniques

No.	Particulars (case No.)	Age (years)	Groups	Chromosome No.	Chromosomal abnormalities identified by G-banding and SKY				Total
					Deletion	Translocation	Inversion	Mosaic	
1	PCP001	61	III	3	3	−	1	−	4
2	PCP002	48	I	13	1	−	2	−	3
3	PCP003	52	II	3, 7	−	1	−	1	2
4	PCP004	59	II	5, 9	2	−	−	1	3
5	PCP005	60	II	7, 9	3	1	1	−	5
6	PCP006	77	IV	1, 8, X	2	3	−	−	5
7	PCP007	55	II	7	1	1	−	−	2
8	PCP008	64	III	3, 9, 13	2	−	1	1	4
9	PCP009	63	III	9, 18	−	2	1	−	3
10	PCP010	49	I	13	1	−	1	−	2
11	PCP011	52	II	7	2	−	−	−	2
12	PCP012	54	II	9	−	1	2	−	3
13	PCP013	75	IV	3, 5, 9, X	5	1	−	1	7
14	PCP014	58	II	5	−	−	−	2	2
15	PCP015	68	III	1, 8	3	1	−	−	4
16	PCP016	45	I	13	2	−	−	−	2
17	PCP017	69	III	1, 9	1	2	−	3	6
18	PCP018	61	III	3, 13	−	4	1	−	5
19	PCP019	60	II	13	−	−	2	−	2
20	PCP020	59	II	3	2	1	−	−	3
21	PCP021	63	III	9, 18	−	2	−	−	2
22	PCP022	68	III	7, 9	2	2	−	1	5
23	PCP023	64	III	13	2	−	1	−	3
24	PCP024	58	II	9	−	1	2	−	3
25	PCP025	72	IV	3, 7, 9, X	6	3	−	1	10
26	PCP026	67	III	1, 18	2	1	−	1	4
27	PCP027	41	I	7	−	1	−	−	1
28	PCP028	54	II	3, 5	−	2	−	1	3
29	PCP029	70	III	1, 8	3	1	1	−	5
30	PCP030	49	I	7, 13	1	1	−	2	4
31	PCP031	63	III	3, 8	−	2	1	−	3
32	PCP032	66	III	13, 18	2	2	1	−	5
33	PCP033	79	IV	1, 6, X	9	4	1	−	14
34	PCP034	60	II	9	2	1	−	−	3
35	PCP035	68	III	3, 9	4	−	1	1	6
36	PCP036	55	II	3	−	−	2	−	2
37	PCP037	62	III	1, 7	−	1	−	2	3
38	PCP038	68	III	8, 13	3	1	−	1	5
39	PCP039	70	III	1, 9	4	2	−	−	6
40	PCP040	58	II	7	1	−	−	1	2
41	PCP041	63	III	3, 8	−	3	−	−	3
42	PCP042	70	III	1, 7	1	3	2	1	7
43	PCP043	52	II	9	−	−	2	−	2
44	PCP044	61	III	3, 13	1	−	3	−	4
45	PCP045	72	IV	5, 8, X	3	3	−	1	7

G-banding: Giemsa-banding; SKY: Spectral karyotyping; PCP: Prostate cancer patients

Table 2 Chromosomal abnormalities in controls identified by sequential G-banding and SKY techniques

No.	Particulars (case No.)	Age (years)	Groups	Chromosome No.	Chromosomal abnormalities identified by G-banding and SKY				Total
					Deletion	Translocation	Inversion	Mosaic	
1	CS001	63	III	5	1	−	−	−	1
2	CS002	45	I	0	−	−	−	−	0
3	CS003	54	II	0	−	−	−	−	0
4	CS004	59	II	2	1	−	−	−	1
5	CS005	59	II	7	−	1	−	−	1
6	CS006	78	IV	2	1	2	−	−	3
7	CS007	55	II	0	−	−	−	−	0
8	CS008	64	III	15	−	1	−	1	2
9	CS009	63	III	12	1	−	−	−	1
10	CS010	48	I	0	−	−	−	−	0
11	CS011	52	II	3	−	1	−	−	1
12	CS012	54	II	0	−	−	−	−	0
13	CS013	74	IV	5	1	−	2	−	3
14	CS014	58	II	0	−	−	−	−	0
15	CS015	67	III	1	1	1	−	−	2
16	CS016	45	I	0	−	−	−	−	0
17	CS017	67	III	7	1	−	−	−	1
18	CS018	63	III	4	−	−	1	−	1
19	CS019	60	II	0	−	−	−	−	0
20	CS020	58	II	0	−	−	−	−	0
21	CS021	63	III	16	−	2	−	−	2
22	CS022	66	III	0	−	−	−	−	0
23	CS023	65	III	2	1	−	1	−	2
24	CS024	58	II	0	−	−	−	−	0
25	CS025	72	IV	13	2	−	−	−	2
26	CS026	66	III	0	−	−	−	−	0
27	CS027	40	I	1, 9	2	1	−	−	3
28	CS028	54	II	4	−	−	−	1	1
29	CS029	70	III	8	−	1	−	−	1
30	CS030	47	I	5	−	1	−	−	1
31	CS031	63	III	6	−	−	1	−	1
32	CS032	68	III	10	−	2	−	−	2
33	CS033	79	IV	2, 14	3	1	−	−	4
34	CS034	60	II	1	−	1	−	−	1
35	CS035	66	III	0	−	−	−	−	0
36	CS036	54	II	5	−	−	1	−	1
37	CS037	61	III	0	−	−	−	−	0
38	CS038	67	III	0	−	−	−	−	0
39	CS039	70	III	8	−	1	−	1	2
40	CS040	58	II	0	−	−	−	−	0
41	CS041	63	III	0	−	−	−	−	0
42	CS042	69	III	0	−	−	−	−	0
43	CS043	52	II	6	−	1	−	−	1
44	CS044	61	III	9	1	−	−	−	1
45	CS045	72	IV	0	−	−	−	−	0

G-banding: Giemsa-banding; SKY: Spectral karyotyping; CS: Control samples

In the present study, chromosome 1 showed the deletion and translocation in prostate cancer patients, groups III and IV showed more number of CA compared to other groups, and group IV particularly had an increase in the number of translocations. The above results of the present study have been supplemented with several reports relating to chromosome 1. Chromosome 1 is involved in prostate cancer whether through the presence of prostate cancer susceptibility genes or through the disruption of common pathways involved in cancer development. Lundgren *et al*.(1992) reported structural chromosomal changes in prostatic tumor tissues. Carpten *et al*.(2002) reported recently that mutations on the *RNASEL* gene (a tumor suppressor gene) on chromosome 1 were responsible for a small fraction of all prostate cancer cases, particularly the most aggressive phenotypes.

In the present study, maximum numbers of CA were deletion, translocation and inversion in the short arm (3p) of chromosome 3 of groups II, III and IV prostate cancer patients. In comparison to groups III and IV, group II showed additional CA in the form of inversions along with deletion and translocation. These results were supported by other studies on chromosome 3. Deletions of the short arm of chromosome 3 (3p24-26 and 3p22-12) were identified in over half of primary prostate cancer cases through LOH (loss of heterozygosity) studies. These deletions were not related to the stage or grade of the tumor (Dahiya *et al*., 1997). Amplification of chromosome 3q25-27 in primary prostate cancer was identified by CGH (comparative genomic hybridisation), Southern blot and comparative PCR (polymerase chain reaction) (Sattler *et al*., 2000).

Chromosome 5 showed deletion and mosaic in prostate cancer patients of the present study. Groups II and IV showed the most number of CA compared to control group. Similar studies have reported the loss of alleles of chromosome 5 was detected in a subset of advanced-stage prostate cancer (Cunningham *et al*., 1996). 5q31-33 was strongly associated with aggressiveness of prostate cancer in a linkage analysis.

In the present study, chromosome 6 showed deletion and translocation in long arm (6q) of prostate cancer patients. This was prominent in group IV patients and controls showed low number of CA in chromosome 6. Other evidences of chromosome 6 involvement in prostate cancer also supported the present study. Almost a third of prostate cancer cases showed LOH as a consequence of deletion of the long arm of chromosome 6, particularly in 6q14-21 (Srikantan *et al*., 1999). The frequency of 6q deletion in invasive prostate cancer was fivefold higher than in organ-confined prostate cancer (Srikantan *et al*., 1999). Chromosome translocation involving chromosome 6 was identified in LNCaP cells, where the breakpoint occurred within the *TPC* gene (homologous to the prokaryotic s10 ribosome protein gene).

Chromosome 7 showed deletion, translocation, inversion and mosaic in prostate cancer patients of the present analysis. Regarding chromosome 7, 7q karyotype was found in all groups and a few of the control group also showed them. Cytogenetic study and FISH studies indicated a gain of chromosome 7 in a substantial number of prostate cancers (Alers *et al*., 1995; van Dekken *et al*., 2000), and this gain was associated with higher tumour grade, advanced pathological stage and early prostate cancer death (described as a high Gleason score) (Takahashi *et al*., 1995). PCR analysis of LOH on prostate cancer indicated a common focus of deletion mapping to 7q31.1-31.2 (Zenklusen *et al*., 1994).

In chromosome 8, the results identified were similar to chromosome 1 observations. FISH or quantitative PCR showed that allelic imbalance occurred at either the distal terminus of the short arm (8p23) or bordered at 8p22 in almost half of primary prostate cancers (MacGrogan *et al*., 1994). The 8p22 deletion appeared to accumulate in advanced stages of prostate cancer.

In the present investigation chromosome 9 showed deletion, translocation and inversion in prostate cancer patients of groups II, III, and IV mainly in the long arm (9q). These results were further strengthened by other reports. Loss of chromosome 9 was identified in prostate cancer using FISH. Aneuploidy of chromosome 9 is associated with recurrent disease. Gain of chromosome 9q was found frequently in primary prostate cancer. A high frequency of deletions in 9q21 was also reported in primary prostate cancer (Perinchery *et al*., 1999).

Chromosome 13 showed deletion and inversion in prostate cancer patients in all groups. Likewise other examples correlating chromosome 13 to prostate cancer include LOH involving the long arm of

chromosome 13 in as many as a third of prostate cancers (Cooney *et al*., 1996; Li *et al*., 1998). Microsatellite marker analysis indicated that loss of *BRCA2* was relatively uncommon in localized prostate cancer but deletion of *Rb* was more frequent in long arm of chromosome 13 (Li *et al*., 1998). LOH of 13q was shown to correlate prostate cancer progression (Afonso *et al*., 1999). Further studies indicated that allelic loss on chromosome 13q14, q21-22 and q33 occurred frequently in metastatic prostate cancer and a subset of prostate cancer (Dong *et al*., 2001).

The present study also noted deletion and translocation in chromosome 16 in prostate cancer patients in both the long and short arms of groups I, III and IV. An increased rate of LOH was initially identified in chromosome 16q (Kunimi *et al*., 1991). Structural abnormalities and trisomies of chromosome 16 in prostate cancer were also identified through karyotyping (Miyauchi *et al*., 1992). FISH analysis mapped the deletions in the long arm of chromosome 16 to 16q22.1-22.3, 16q23.2-24.1 and 16q24.3-ter, encompassing E-cadherin, a putative tumour suppressor gene mapped to 16q22.1.

The present study showed CA like deletion, translocation in the long arm of chromosome 18, in groups III and IV. Deletion of the long arm of chromosome 18 was observed in over 40% of primary prostate cancer (Kunimi *et al*., 1991; Ueda *et al*., 1997). LOH was frequently found in chromosome 18q21 (Ueda *et al*., 1997). Deletion of chromosome 18 occurs more extensively in a metastatic type of prostate cancer.

In chromosome X, deletion and translocation were identified in this study. In group IV, maximum number of the above CA type was observed. Numerical abnormality of the chromosome X was reported in 40% of prostate cancer (Miyoshi *et al*., 2000). An increased copy number of chromosome X was associated with hormone-refractory prostate cancer, albeit with a high intra tumor heterogeneity (Koivisto *et al*., 1995).

In the present investigation, increasing age is directly proportional to the increase in CA, and this may also include factors like hormonal inter play, environmental and life style. But age presents a convincing evidence for an increase in the number of aberrations which may be correlated to the onset of this cancer type. In the same way, the study by Sakr *et al*.(1994) involving the autopsy series of prostate cancer patients revealed small prostatic carcinomas in up to 29% of men aged 30 to 40 years and 64% of men of 60 to 70 years old.

CONCLUSION

This study showed that chromosomal instability in peripheral blood lymphocytes increased in an age wise manner in prostate cancer patients, suggesting the role of age in the development of prostate cancer. CA may hence prove to be a potential biomarker for prostate cancer susceptibility. While the molecular genetic events associated with the initiation and progression of prostate cancer remains poorly understood, it has long been considered that genetic instability plays a pivotal role in the development and progression of human cancer (Loeb, 1991), and as with most types of human cancer, multiple genetic changes are probably necessary for prostate carcinogenesis.

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