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Advances in Brief

Prevalence of Androgen Receptor Gene Mutations in Latent Prostatic Carcinomas from Japanese Men[1]

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Abstract

The incidence rate of clinically apparent prostatic carcinoma is 8-fold higher in the United States than in Japan, while the prevalence of latent prostatic carcinoma, a presumed precursor to clinical carcinoma, is similar in the two countries. The purpose of this study was to investigate the hypothesis that this profound difference in incidence rates of clinical carcinoma reflects distinct profiles of molecular genetic alterations in the latent precursor lesions that occur in the two countries. A significant fraction of latent carcinomas from Japanese men were found to contain inactivating mutations of the androgen receptor gene, while no such mutations were found in latent carcinomas from American men. No mutations were found in clinical carcinomas from either country. These data offer a potential molecular genetic explanation that may partially account for the distinct prostatic carcinoma incidence rates in these two populations.

Introduction

Carcinoma of the prostate is the most commonly diagnosed cancer and the second leading cause of cancer mortality in American males (1). The incidence of this malignancy is widely variable among populations throughout the world; the incidence rate for Japanese is approximately 8-fold lower than that for Americans (2). In contrast, the prevalence of subclinical prostate cancer identified at autopsy, known as latent or histological prostate carcinoma, is similar among Americans and Japanese (3–6). These observations contribute to the uncertainty regarding classification of latent carcinoma as a relatively indolent variant of prostatic carcinoma or, alternatively, a carcinoma that would have manifested clinically if the patient had lived longer (7, 8).

Empirical evidence is most consistent with the hypothesis that only certain latent tumors become clinically apparent, the others remaining clinically undetected having accumulated some but not all of the molecular genetic alterations necessary for them to become more aggressive and clinically detectable, or perhaps having a distinct molecular genetic character (9). Thus, it would follow that the discrepancy between American and Japanese clinical cancer incidence results from differential environmental or life-style factors affecting the progression of latent to clinical carcinoma or, alternatively, that the latent tumors from these populations are characterized by distinct molecular genetic features, leading to a higher frequency of progression to clinical carcinoma in Americans than in Japanese. Consistent with the latter possibility is a study in which mutations of the *RAS* proto-oncogene were found to be common in latent tumors from Japanese (10); although this study did not include tumors from Americans, it has been well documented elsewhere that *RAS* mutations are rare in prostate carcinomas from Americans (11, 12).

In this study, we explore further the hypothesis that distinct molecular genetic alterations characterize prostate cancers from Americans and Japanese. The androgen receptor gene was chosen for this analysis based on the integral role of its encoded protein in prostate growth and differentiation, as well as previous observations that mutations of this gene may occur in clinical prostate carcinomas (13–16). A direct four-way comparison was made between latent and clinically apparent prostate cancers from American and Japanese men.

Materials and Methods

Tissue Acquisition and DNA Preparation. A total of 181 tumors were retrieved for this study, all as formalin-fixed, paraffin-embedded tissue specimens from pathology archives. Tumors from Japanese men consisted of 74 latent prostatic carcinomas and 38 clinical prostatic carcinomas and were obtained from the Department of Pathology at Jikei University School of Medicine, Tokyo, Japan. Tumors from American men consisted of 43 latent prostatic carcinomas and 26 clinical prostatic carcinomas and were obtained from the Department of Pathology at the Medical College of Georgia, Augusta, Georgia. Hematoxylin and eosin-stained slides from each case were reviewed by the study pathologists (M. F. and W. C. A.) to confirm the original diagnoses of latent or clinical prostatic carcinoma. Stained slides were used as a guide to separate tumor and normal tissues by microdissection using 10 μm-thick unstained tissue sections on glass slides. Sterile techniques were used to avoid cross-contamination between tissue specimens. Following deparaffinization with xylenes and ethanol, tissues were digested in TE buffer (pH 8.0) containing 0.5% SDS and 500 μg/ml of proteinase K for 3 days, with additional proteinase K added every 24 h. Following a standard series of phenol/chloroform extractions, DNA was precipitated and resuspended in TE buffer.

SSCP[3] Analysis. Intron-based PCR primers were designed to amplify individual exons B-H of the androgen receptor gene, based on the published sequence (17). PCR reactions were carried out in a volume of 20 μl containing 50 ng of genomic DNA, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 200 μM each of dATP, dGTP, and dTTP, 20 μM dCTP, 2 μCi of [α-^{32}P]dCTP (3000 Ci/mmol, Amersham), each primer at 1 μM, and 1 unit of Taq polymerase (Perkin-Elmer). Amplification was for 35 cycles in a Perkin-Elmer 480 thermal cycler, each consisting of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C, followed by a 7-min extension at 72°C following the last cycle. A 4-μl aliquot of the PCR product was diluted into 40 μl of denaturing loading buffer [95% formamide, 10 mM EDTA (pH 8.0), 0.2% xylene cyanol FF, and 0.02% bromophenol blue], heated at 95°C for 5 min, and cooled on ice; 5 μl of this solution were loaded for electrophoresis. Gels for SSCP analysis consisted of 0.5X MDE solution (AT Biochem, Malvern, PA) and 0.6X TBE buffer and were run in 0.6X TBE buffer at 8W for 16–18 h at room temperature.

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[3] The abbreviation used is: SSCP, single-strand conformation polymorphism.

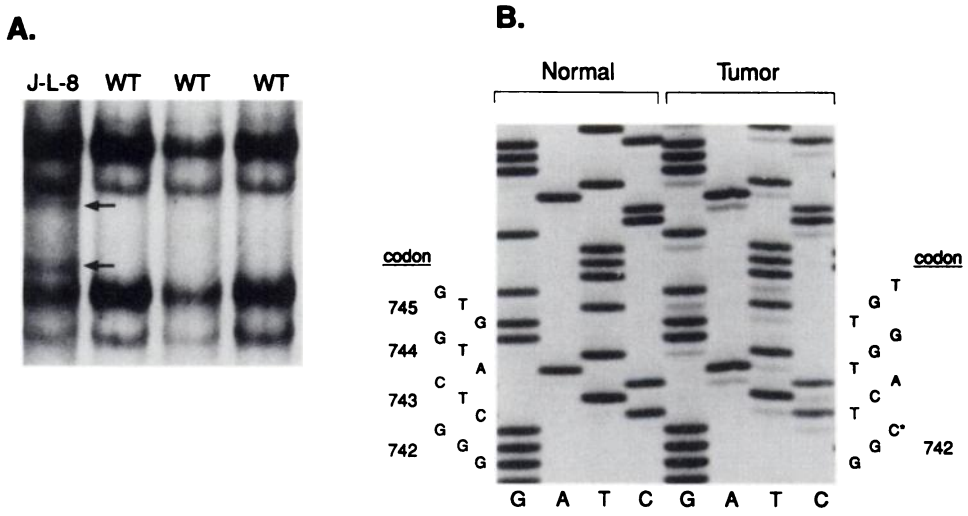

Fig. 1. Deletion mutation in a Japanese latent prostatic carcinoma. *A*, SSCP analysis of exon E showed two bands of altered mobility (*arrows*) in DNA from tumor JL8. *WT*, wild-type. *B*, sequence analysis of exon E PCR product from normal genomic DNA (normal) and tumor JL8 genomic DNA (tumor) revealed a single base deletion at codons 742/743, causing a frameshift in tumor DNA. Overlapping wild-type sequence results from DNA of normal cell infiltration of the tumor.

Following electrophoresis, gels were dried and exposed to Hyperfilm MP (Amersham) for 3–18 h at room temperature.

Sequence Analysis. Following autoradiography for SSCP analysis, variant and wild-type bands were excised from the gels and eluted into 50 μl of TE buffer at 37°C for 2 h. Two μl of the eluted DNA were used as template for PCR amplification under conditions identical to those described above, with the exception that all deoxynucleotide triphosphates were at 200 μM and radiolabeled dCTP was omitted. Genomic DNA from the corresponding tissue sample was PCR amplified in parallel with the isolated bands. The PCR products were electrophoresed in low melting point agarose, excised from the gel, purified using the Wizard PCR Prep DNA Purification System (Promega), and resuspended in TE. An aliquot of this DNA sample was subcloned into pBluescript SK+ (Stratagene), and approximately 80 pooled clones were sequenced for each DNA sample using Sequenase 2.0 (U.S. Biochemical), and the same primers were used for PCR amplification. Three μl of each sequencing product were diluted into 9 μl of denaturing loading buffer (as described above) and heated at 70°C for 5 min; then 3 μl were loaded into 6% polyacrylamide gels containing 8.3 M urea. Electrophoresis was at 70 W for 3–4 h at room temperature. Gels were fixed in 10% methanol/10% glacial acetic acid, dried, and exposed to Hyperfilm MP for 1–3 days at room temperature.

Results

Exons B-H of the androgen receptor gene were screened for mutations in 186 tumor specimens using SSCP analysis. No variants were identified in clinically diagnosed prostatic carcinomas from American (n = 26) or Japanese (n = 38) men. Among latent carcinomas, no variants were found in those from American men (n = 43), but in 79 latent tumors from 74 Japanese men, a total of 18 variants were identified. Sequence analysis of the variant products revealed four frameshift mutations, four nonsense mutations, and 10 missense mutations. Examples of each of these types of mutations are shown in Figs. 1–3. All of these mutations were somatically acquired and tumor specific, as SSCP and sequence analyses of DNA from corresponding normal tissues revealed the wild-type sequence in every case. The presence of sequence from a normal allele in the tumor lane is from the DNA of normal stromal or glandular cells interspersed with the infiltrating tumor. All of the frameshift mutations involved a single base pair deletion, three of which clustered within a four-codon region of exon B. All of the nonsense mutations occurred in either of two closely spaced codons encoding a tryptophan in exon E. The missense mutations were spread throughout exons E, G, and H. Of the 14 missense and nonsense mutations, all were transitions. A summary of the mutations identified in Japanese latent carcinomas is presented in Table 1.

Discussion

A mutation analysis of the androgen receptor gene revealed no alterations in 64 clinical prostatic carcinomas from American and Japanese men. Remarkably, however, 18 mutations were identified in 79 latent carcinomas from Japanese men, while none were found in 43 similar tumors from American men. The results of this study are not inconsistent with those of four previous reports, suggesting that androgen receptor gene mutations are rare in clinical prostatic carcinomas, as a total of only six mutations have been described (13–16). While these studies have included clinically diagnosed early and

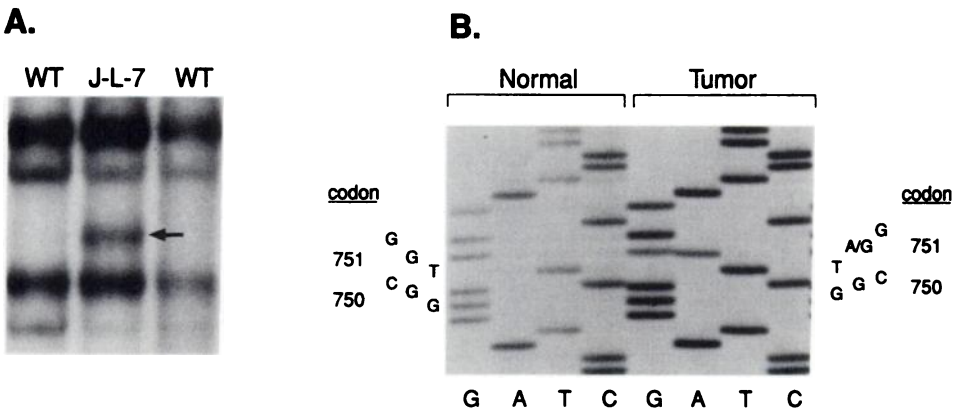

Fig. 2. Nonsense mutation in a Japanese latent prostatic carcinoma. *A*, SSCP analysis of exon E showed a band of altered mobility (*arrow*) in DNA from tumor JL7. *WT*, wild-type. *B*, sequence analysis of exon E PCR product from normal genomic DNA (normal) and tumor JL7 genomic DNA (tumor) revealed a point mutation at codon 751 changing a tryptophan to a premature termination codon. Presence of wild-type sequence results from DNA of normal cell infiltration of the tumor.

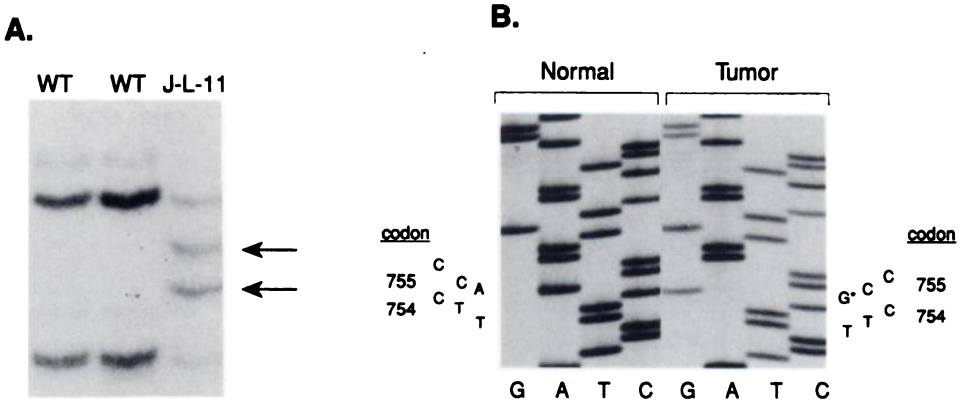

Fig. 3. Missense mutation in a Japanese latent prostatic carcinoma. *A*, SSCP analysis of exon E showed two bands of altered mobility (*arrows*) in DNA from tumor JL11. *WT*, wild-type. *B*, sequence analysis of exon E PCR product from normal genomic DNA (normal) and shifted SSCP band from tumor JL11 (tumor) revealed a point mutation in codon 755, changing a threonine to an alanine.

Table 1 *Androgen receptor gene mutations*

No.	Tumor	Exon	Codon[a]	Mutation	Result	Type
1	JL17	B	547	TTG → TT	Leu → frameshift	Deletion
2	JL63	B	554	CCA → CC	Pro → frameshift	Deletion
3	JL42	B	554	CCA → CC	Pro → frameshift	Deletion
4	JL49	E	741	TGG → TAG	Trp → stop	Nonsense
5	JL8[b]	E	743	GGG → GG	Gly → frameshift	Deletion
6	JL9[b]	E	744	CTC → TTC	Leu → Phe	Missense
7	JL15	E	748	GCC → GTC	Ala → Val	Missense
8	JL38	E	749	ATG → ATA	Met → Ile	Missense
9	JL33	E	750	GGC → AGC	Gly → Ser	Missense
10	JL7	E	751	TGG → TAG	Trp → stop	Nonsense
11	JL19	E	751	TGG → TGA	Trp → stop	Nonsense
12	JL72[c]	E	751	TGG → TAG	Trp → stop	Nonsense
13	JL72[c]	E	754	TTC → CTC	Phe → Leu	Missense
14	JL11	E	755	ACC → GCC	Thr → Ala	Missense
15	JL53	E	759	TCC → CCC	Ser → Pro	Missense
16	JL28	E	763	TAC → TGC	Tyr → Cys	Missense
17	JL21	G	865	GTG → ATG	Val → Met	Missense
18	JL46	H	909	GGG → GAG	Gly → Glu	Missense

[a] Reading frame numbered according to Lubahn *et al.* (17).
[b] Tumors JL8 and JL9 are independent tumors from the same individual.
[c] Two mutations were identified in the same tumor specimen.

advanced stage tumors from both American and Japanese men, we are unaware of any study in which latent prostatic carcinomas were examined for androgen receptor gene mutations. The results described herein suggest that such mutations, while present only in a minority of the tumors examined, are nevertheless significantly more common in latent tumors from Japanese than from American men and have implications regarding the striking epidemiological disparities in prostatic cancer incidence in these two countries.

The majority of the genetic alterations that were identified are predicted to inactivate the androgen receptor protein. The eight frameshift and nonsense mutations would result in premature truncation of the full-length protein. The 10 missense mutations occur in highly conserved regions of the hormone binding domain that, when mutated, have been associated with androgen insensitivity syndrome (18–22). Furthermore, location of the androgen receptor gene on chromosome X confers a hemizygous state in males, increasing the probability of phenotypic consequences following mutational inactivation of the gene.

The nature of the mutations reported here is also noteworthy, in that all 14 of the missense and nonsense mutations were G:C to A:T (72%) or A:T to G:C (28%) transitions. Of the 10 G:C to A:T transitions, only three of these mutations occurred at CpG sites. Taken together, the clustering of deletion and nonsense mutations to particular regions of the gene, the prevalence of transition mutations, and the common occurrence of mutations in latent carcinomas from Japanese men compared to the other tumor categories studied imply that factors other than simple random mutagenesis followed by clonal selection are operative in generating the mutations in these tumors. The occurrence of two different mutations in one tumor and two different mutations in two coexistent tumors from the same individual (Table 1) underscore this hypothesis. Unique environmental or dietary exposures in the Japanese population would seem to represent plausible etiological influences, but no direct evidence for such a relationship currently exists.

Previous studies have focused discussion on the potential role of androgen receptor mutations in contributing to the hormone insensitivity phenotype displayed by many recurrent or advanced-stage prostatic carcinomas (23). Our results, however, suggest another mechanism through which mutations of this gene may contribute to the behavior of prostatic malignancies. These mutations may represent a molecular genetic dead-end for latent prostatic carcinoma, effectively blocking further cellular expansion and genetic progression toward clinical manifestation. Interruption of the androgen receptor-mediated pathway of proliferation and differentiation through receptor inactivation would be predicted to have the same ultimate consequence as androgen ablation therapy for hormone-dependent tumors, programmed cell death (24). Further studies of latent prostatic tumors might thus be expected to reveal a higher frequency of apoptotic lesions from the Japanese population compared to those from Americans. Nevertheless, these latent tumors appear to represent clonal expansion of cells with the androgen receptor gene mutations. Therefore, these mutations do not immediately eliminate the altered cells. The possibility exists that cells with androgen receptor gene mutations have a selective advantage early in the development of these cancers and a subsequent disadvantage in the later stages of malignant progression.

These results are consistent with the hypothesis that a lower clinical carcinoma incidence rate in Japanese, as compared to Americans, may partially result from a higher rate of androgen receptor gene mutation in latent tumors, thus halting their further malignant progression. Although these findings cannot account for the 8-fold difference in clinical prostatic cancer incidence rates between these two countries, they may account for some of this difference. The fact that mutations were found in only about one-quarter of the Japanese latent tumors examined may reflect an underestimate of the mutation prevalence as a result of mutation screening insensitivity. Alternatively, other perturbations in the androgen receptor pathway may be involved in the tumors without androgen receptor gene mutations. It is not clear how inactivating mutations of the androgen receptor gene would be susceptible to clonal selection under this scenario, but further studies of this phenomenon should help clarify the significance of these observations.

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