RESEARCH COMMUNICATION

Role of an Androgen Receptor Gene Polymorphism in Development of Hormone Refractory Prostate Cancer in Indian Population

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Abstract

Background: Androgen receptors play critical roles in the development of primary as well as advanced hormone-refractory prostate cancers. Since the growth of prostate cancer is androgen-sensitive, metastatic disease has been treated by hormonal therapy in the form of androgen ablation. Prostate cancer cells rely on androgen receptor (AR) for proliferation and survival. Aim: To evaluate the prognostic significance of androgen receptor polymorphism in patients under hormonal therapy in any form. Methods: Complete follow up data were available for 87 patients out of 130 patients enrolled for study. DNA was extracted from blood samples using salting out method and then subjected to PCR Genscan for CAG and GGN genotyping. The mean follow up was 10.12±8.83 months. Results: Out of 87 patients, 64 experienced clinical as well as biochemical recurrence. The overall hormone refractory rates were 73.4% after one year. We observed a significant shorter median CAG repeats in HRPC patients (20 vs 22). The hazard ratio for HRPCs with the ≤20 CAG repeat genotype was 0.602 (0.33-1.08, p=0.09). Kaplan-Meier analysis showed that HRPC rates were not significantly associated with CAG repeat (p=0.06) but a trend was observed with short CAG repeats. No significant association was observed with AR-GGN repeats. Conclusions: A trend for association of AR-CAG repeats with HRPC patients in north Indian population was observed, suggesting this to be a prognostic factor for determining the therapeutic regimen.

Key words: Androgen receptor - hormone refractory prostate cancer - North Indian population

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Introduction

Prostate cancer is the third most common cancer in the world and the most frequently diagnosed male cancer in western countries (Ferlay et al., 2001). However, in Asia, the incidence of prostate cancer is significantly lower and it often plays second fiddle to lung, stomach and colon cancer. In India, it is the sixth most common cancer among men (Sinha et al., 2003). Variation in incidence and mortality rate from prostate cancer around the world suggests that it is multifactorial and polygenic in origin. In western countries due to screening there is a significant stage migration so approximately 80% of the patients are detected in early stage. The reverse is in our country where we see more than 90% patients present in advanced stage.

The molecular events involved in neoplastic intiation and progression is poorly understood, despite the recognition of various events during prostate cancer tumorigenesis. Polymorphic variations in humans may be responsible for inter-individual differences in susceptibility to multifactorial diseases. The main spectrum in our clinical practice is of metastasis prostate cancer where the only treatment currently available is androgen derivation therapy. More than 90% of the patients show an initial biochemical response to the therapy (Palmberg et al., 2000), and clinical response rates of 80% have been reported (Gittes, 1991). However, during the therapy, the hormone-refractory tumor cells eventully emerge leading to clinical progression.

During the development of the normal prostate and of prostate cancer, cell survival depends primarily on the androgen receptor mediating the effect of androgens. In the nucleus, the androgen receptor dimerizes, binds to androgen-response elements in DNA, and activates the transcription of genes involved in the growth and survival of the cell. In line with this it has been suggested that polymorphisms in exon 1 of the AR gene, especially the length of CAG and GGN repeat, are associated with the risk of prostate cancer. Transactivation efficiency of AR increases with short CAG repeat and it regulates the gene transcription by binding to androgen response elements (ARE) to promoter of target genes such as PSA, KLK-2, CDK2 and p16 (Sun et al.,1997). The present study was therefore undertaken to assess the role of AR polymorphism in development of hormone refractory prostate cancer.

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Materials and Methods

The retrospective study included consecutive North Indian patients (n =130) with histologically confirmed prostate cancer (CaP) during January 2003 to November 2005 from Department of Urology of the Institute. The ethical committee of our institute approved the protocol and the study. Patients of advanced and metastatic prostate cancer requiring androgen ablation therapy in any form were assessed every 3 months for the first year, every 6 months for the second year and every year thereafter clinically, biochemically with a PSA test, and radiographically as indicated. The primary endpoint was HRPC defined as a documented clinical progression or bone scan or soft tissue metastasis and/or rise in serum PSA of nadir level on two consecutive measurements taken at least three months apart (Bubley et al., 1999). Each individual was asked to provide blood samples for PSA and genotyping analysis. Of the 130 patients, complete follow up data was available of only 87 (64.4%) patients.

PCR Assay for AR Polymorphism

Genomic DNA was isolated from blood leukocytes by Proteinase-K digestion and Phenol/Chloroform method (Miller et al., 1988). Exon 1 of AR gene was genotyped using the primers flanking the CAG repeat region: forward, 5'-CAGAATCTGTTCCAGAGCGTGC- 3'; reverse, 5'-AAGGTTGCTGTTCCTCATCCAG- 3' (Cram DS, et al. 2000). Primers were synthesized in an ABI 392 oligosynthesizer (Perkin Elmer, Foster City, Calif.). The forward primer was synthesized with 5'FAM (Carboxy-fluorescein) label (Perkin Elmer) in order to analyze the PCR product in the automated DNA sequencer (ABI 377). Each PCR was carried out in a total volume of 10µl containing 10ng DNA, 1X PCR buffer containing 1.5 mM MgCl2, 10pM of each primer, 200µM deoxynucleotide triphosphates and 2U of AmpliTaq Gold (Perkin Elmer). Thirty PCR cycles were performed each consisting of 1min at 94°C, 1 min at 60°C and 1 min at 72°C followed by a final extension at 72°C for 5min.

One microlitre of PCR product was mixed with 1.5µl of loading dye (Formamide:blue dextran; 5:1), 0.5µl of GS-ROX500 (0.5µl/sample) and denatured at 94°C for 2 minutes. Samples were electrophoresed in 5% Long Range (FMC) gel using an ABI 377 automated DNA sequencer (Perkin Elmer) at Centre for Cellular and Molecular Biology, Hyderabad, India. Raw data were analysed using GeneScan and Genotyping software (Perkin Elmer) to obtain the allele (repeat) size.

Statistical Analysis

Statistical analysis was done with SPSS software 11.5. For androgen receptor analysis the mean and median number of repeats were compared between different groups using non-parametric tests. The effects of the number of repeats of the androgen receptor gene in predicting the treatment outcome or cancer progression were examined by univariate and multivariate Cox proportional hazard modeling. Kaplan-Meier survival estimates were done to compare the survival curves of different repeat groups.

Results

The mean follow up was 10.1±8.8 months (2-36 months). Out of 87 patients, 64 (73.6%) experienced clinical as well as biochemical recurrence (HRPC) and 49/87 (56.3%) expired till the last date of follow up. The overall hormone refractory (or androgen dependent survival) rates were 73.4% after one year and 26.6% at more than one year.

Mean PSA of HRPC patients were significantly higher than the PSA in non-HRPC patients (239.1 vs 123.1 ng/ml). Median test (non-parametric analysis) also showed a significant difference in the PSA level between two groups (p=0.006). Similarly the patients who were dead during the follow up were having comparatively higher serum PSA level than those who survived (155.4 vs 263.9 ng/ml), although it was non-significant. The median number CAG repeats for patients who had HRPC and patients who did not have HRPC were 20 and 22 respectively (p=0.026). Similarly mean CAG repeat of HRPC patient was also significantly lower as compared to those patients without HRPC (19.9±3.7 vs 22.4±3.6). Similarly, The patients expired within the follow up period also demonstrated a significantly lower mean and median CAG repeat (19.5±3.8 vs 21.9±3.5 p=0.003; 19.5 vs 21.5 p=0.025).

The hazard ratio for development of hormone

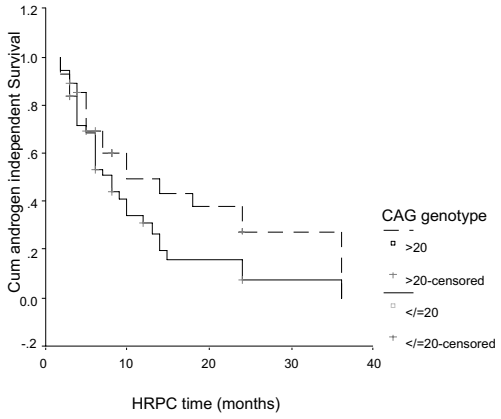

Figure 1. Kaplan-Meier Recurrence-Free Survival for Patients with two CAG Genotypes: ≤20 CAG and >20 CAG

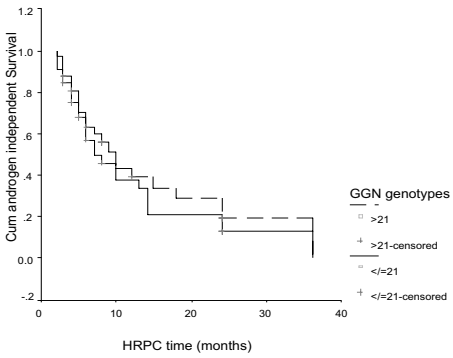

Figure 2. Kaplan-Meier Recurrence-Free Survival for Patients with two GGN Genotypes: ≤21 GGN and >21 GGN

refractory prostate cancer with the 20 CAG repeat genotype, compared with patients with the >20 CAG repeat genotype was 0.602 (95% CI =0.33-1.08; p=0.09). The median time for HRPC development in patients with the 20 CAG repeat genotype was 8 months while with those having CAG repeat >20, it was 10 months (p>0.05). Kaplan-Meier curve analysis showed that HRPC rates were not statistically associated with number of AR-CAG repeat (p=0.06) but a trend of greater event was observed in predicting the recurrence of the disease (Figure 1) with short CAG genotype.

In case of AR-GGN repeat analysis, no significant difference was observed in mean GGN repeats between HRPC patients and non-HRPC patients (21.22±2.1 vs 21.52±2.2; p=0.992). Median GGN repeat was similar in both the groups (21 repeats).

The hazard ratio for development of hormone refractory prostate cancer with the 21 GGN repeat genotype was 1.12 (95% CI =0.69-2.16; p=0.486) as compared with those having >21 GGN repeat genotype. The median time for HRPC development in patients with the 21 GGN repeat genotype was 7 months while with those having CAG repeat <21, it was 10 months (p>0.05). Kaplan-Meier curve analysis showed that HRPC rates were not statistically associated with number of AR-GGN repeat (p=0.449). Thus, it showed that the length of GGN repeat was not a significant factor in predicting the recurrence of the diseases (Figure 2).

Discussion

Androgen receptor (AR) is a ligand dependent steroid hormone transactivation factor. Its trans-activity is regulated by androgens, which have a critical role in the proliferation and differentiation of prostate cells. Based on these assumptions, Coetzee and Ross (1994) had hypothesized that variation in transcriptional activity of AR, related to polymorphic CAG repeats, influences prostate carcinogenesis. Metastatic prostate cancer is usually treated with androgen suppression, antiandrogens, or a combination of the two (Laufer et al., 2000). Despite an initial response, progression is inevitable, because of the emergence of androgen-independent prostate-cancer cells. In most androgen-independent prostate cancers, expression of the receptor and many aspects of its function are maintained (Feldman et al., 2001). There is evidence that receptors drive the proliferation of androgen-independent prostate-cancer cells even in the absence of androgens (Zegarra-Moro et al., 2002). Many somatic alterations of AR have been detected in prostate cancers, especially in those that progress despite hormonal treatment (Haapala et al., 2001).

We hypothesized that short AR-CAG repeats are associated with shorter time of androgen independent prostate cancer. But our results indicate that the length of the CAG repeat was not a significant factor in predicting the time of HRPC (p=0.06).

The major findings of this study indicated an increased risk of prostate cancer with AR-CAG repeat as significantly short median CAG repeat was observed in hormone refractory prostate cancer (HRPC) patients as compared to non-refractory patients while median GGN repeat was same in both HRPC as well as non-HRPC patients. Overall, androgen receptor (CAG or GGN) repeats were not established as a significant factor in predicting the time of development of androgen independent prostate cancer or HRPC.

The median time of recurrence was 8 and 10 months with short (≤20) and long (>20 CAG) CAG repeats respectively. The median survival was low in our population may be due to lack of structured screening programme for prostate cancer in India. Most of the cases are, therefore, diagnosed at our Institute is at advanced stage of cancer. This trend of higher mortality was in 70s in USA, when PSA screening was not done. Most of the HRPC patients were having very high serum PSA level as compared to others. A previous study by Mononen et al (Mononen et al., 2002) also presented the non-significant association of CAG repeat with recurrence of the disease in Finnish population. Cude et al (2002) also found no association between number of CAG repeats and time for progression of cancer or overall survival time. Few other studies have shown that the CAG repeat was not associated with time for progression after initiation of hormonal therapy (Hardy et al., 1996), disease-free survival or overall survival (Edwards et al., 1999). Bratt et al (Bratt et al., 1999) reported that men with longer CAG polymorphisms had better responses to hormonal therapy after adjusting for baseline PSA, tumor grade and stage. This is in contrast to previous studies, which found relationship between the CAG repeat and other variables of prostate cancer aggressiveness (Hakimi et al., 1997; Bratt et al., 1999). Although we observed a significant difference in median CAG repeats of those patients who become hormone refractory with in 36 months of diagnosis and those who survive without refractory CaP (20 vs 22; p=0.026).

In case of AR-GGN repeats also no significant association was observed with hormone refractory prostate cancer. The median GGN repeats were same in HRPC as well as non-HRPC patients. Till date, to the best of our knowledge there is no study depicting association of HRPC time with AR-GGN repeat numbers in any population. The reason is speculative to be its non significant association with risk of prostate cancer.

Considering these studies, we expect that the CAG repeat length plays a little role in dictating clinical outcomes in prostate cancer patients. AR amplification, accompanied by over expression of androgen receptors, may promote the growth of androgen-independent prostate cancer cells by increasing the sensitivity of prostate-cancer cells to low levels of circulating androgens (Haapala et al., 2001). In the absence of AR mutations, androgen-independent prostate cancer may progress through the activation of ligand-independent androgen-receptor signaling pathways (Nazareth et al.; 1996; Sadar et al., 2000). Further studies perhaps in large cohort of different population and over a long follow up period with HRPC are necessary to better understand the genetic influences on the development and progression of prostate cancer and to possibly integrate this information into a multigenic model for prostate cancer susceptibility.

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