Polymorphisms of Human Androgen Receptor (hAR) Gene in Prostate Cancer Cell Lines PC-EW and PC-OR

DIRK G. ENGEHAUSEN1, F. STEFFEN KRAUSE1, JOHANN FLEISCHMANN1, ZIYA AKCETIN1, KARL M. SCHROTT1 and SABINE ENDELE2

1Department of Urology and 2Institute of Human Genetics, University of Erlangen-Nuremberg, Krankenhausstraße 12, D-91054 Erlangen, Germany

Abstract. Background: Prostate cancer is the leading tumor of the male in Western societies. Genetic alterations of the androgen receptor gene are known in the advanced metastatic disease. In this study, the androgen receptor gene was tested in two human prostate cancer cell lines, the androgen-sensitive PC-EW and the androgen-independent PC-OR. Materials and Methods: Genomic DNA was isolated from two cell lines from metastatic prostate adenocarcinoma in heterotransplanted male athymic nude (nu/nu) Balb/c mice. Mutation screening was performed by sequencing of exons 1-8 of the human androgen receptor gene. Results: Despite two polymorphisms found in the transactivation domain of hAR exon 1, no point mutations were detected in the hAR gene of both cell lines. Conclusion: Point mutations of hAR are not necessary for metastatic prostate cancer, while alterations in the polyglutamine and polyglycine repeat region in exon 1 of the hAR gene are more often found. These repeats are two of many genetic influences that contribute to the overall risk of developing prostate cancer.

Prostate cancer is the leading tumor of the male in Western societies. This disease accounts for nearly 9% of all cancer deaths among men in the USA and Europe. Genetic alterations of the androgen receptor gene are known in the advanced metastatic disease and also in the androgen-independent tumor. The human AR (hAR) gene is located at chromosome Xq11.2-q12 and is more than 90 kb in length (1). The role of hAR gene mutations in carcinogenesis of the prostate was investigated in this study, using two prostate tumor cell lines (the androgen-sensitive PC-EW and the androgen-independent PC-OR), which were isolated from metastatic tumors.

Materials and Methods

The origin of the PC-EW tumor cell line and its establishment and maintenance by serial transplantation in nude mice have been previously described (2-4). The origin of the PC-OR tumor was from lymph node metastases from a human pT3, N2, M0 prostate adenocarcinoma taken by lymph node dissection. Both patients were German Caucasians. The histopathology showed a poorly-differentiated tumor (G3). PC-OR is an androgen-independent carcinoma, described in 2001 (5). None of the carcinomas were treated with chemotherapy prior to surgery or collection of the cells for heterotransplantation.

High molecular weight genomic DNA was extracted from the two cell lines from metastatic prostate adenocarcinoma in heterotransplanted male athymic nude (nu/nu) Balb/c mice according to the manufacturer’s instructions (Qiagen, Hilden, Germany). The "Bagg albino" mice were developed in 1913 by H.J. Bagg from an Ohio pet dealer and inbred in 1923 by McDowell (Charles River Laboratories, product information 2003). Besides the sequences of primers for exon 1, which have been published (9), new oligonucleotide primers were designed to flank the exon/intron boundaries of exons 2-8 of the hAR gene for use in PCR amplification (Table I). PCR reactions were performed in 50 µl volumes using 100 ng of genomic DNA template, 1.0 µM of each primer, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM MgCl2, 200 µM of each deoxynucleotide triphosphate and 0.5 unit of Taq DNA polymerase (Invitrogen, Karlsruhe, Germany). Additionally, 1 M betaine was added for the amplification of exons 1, 7 and 8. The PCR was performed with an initial denaturing step at 95°C for 3 min, then 35 cycles beginning with 94°C for 1 min, followed by an annealing step (57°C for exons 1.1 and 1.2, 55°C for exon 1.3; 57°C for exons 2-6 and 50°C for exons 7 and 8) for 1 min, an elongation step for 1 min and 30 sec and a final extension step at 72°C for 10 min. The PCR products were isopropanol precipitated and both sense and antisense DNA strands of the PCR products were directly sequenced with the ABI PRISM Big Dye™ Terminator v3.0 Cycle Sequencing Ready Reaction kit (Applied Biosystems, Weiterstadt, Germany). The polymorphisms in exon 1 were confirmed in two independent PCR-amplified DNA fragments.

Key Words: Prostate cancer, androgen receptor, PC-EW, PC-OR, mutation screening.
Results

DNA from cell lines PC-EW and PC-OR was analyzed for mutations by amplification of exons 1-8 of the hAR gene and subsequent sequence analysis. Two different polymorphisms were found. The polyglutamine repeat in the transactivation domain of hAR exon 1 in PC-EW had a CAG repeat length of 19, and in PC-OR a CAG repeat length of 22, compared with a normal length of 21 repeats in US Caucasians. In the polyglycine repeat area, we found a GGN repeat length of 17 in PC-EW and 23 in PC-OR, compared with a normal length of 23 repeats in US Caucasians (Figure 1). Thus, the difference from the normal in the repeat length in both polymorphisms in the androgen-sensitive PC-EW is greater than in the androgen-independent PC-OR cell line. Interestingly, we observed a heterogeneity in the repeat length in both polymorphisms in the androgen-sensitive PC-EW which is greater than in the androgen-independent PC-OR cell line. Interestingly, we observed a heterogeneity in the repeat length in both polymorphisms in the androgen-sensitive PC-EW, as well as in PC-OR. A second allele and 16 (PC-EW) and 22 (PC-OR) repeats could be detected in the amplified material. Sequencing of four DNAs extracted from whole blood samples of healthy control persons also showed signs of heterogeneity. This heterogeneity may be explained either by somatic variability or by a PCR artifact.

Discussion

Different hAR gene mutations including point mutations, point insertions and small deletions, mostly in the steroid-binding domain of exon 4 to exon 8, were found in 22-50% of hormone-relapsed prostate cancers (1, 6-9). Two polymorphisms are described in exon 1, in the transactivation domain of hAR gene: the highly polymorphic CAG repeats (polyglutamine region) in the first third of this exon and the moderate polymorphic GGN repeats (polyglycine region) in the last third.

Short CAG repeat lengths have been associated with androgenetic alopecia, ankylosing spondylitis, mental retardation and also with the benign prostatic hyperplasia (BPH) (10, 11). A shorter hAR CAG repeat imposes a higher transactivation activity on the receptor and an increased binding affinity for androgens (12). This may make the prostate more vulnerable to chronic androgen overstimulation and increased proliferative activity, which could increase the rate of somatic mutations among tumor suppressor genes (13). Abnormal extension of the glutamine repeat (38-52 repeats) is associated with spinal and bulbar muscular atrophy (Kennedy Syndrome, SBMA), with reduced fertility, low virilization, reduced sperm production and testicular atrophy (14, 15). Men with SBMA have also been diagnosed with prostate cancer (16). Many studies suggested that there is a positive association between shorter CAG repeats and the occurrence of prostate cancer (17, 18). However, other studies show no statistically significant association between shorter CAG length and the risk of the disease (19). It has also been suggested that the CAG repeat length may be associated with prostate cancer aggressiveness (20).

There are reports suggesting that there is a correlation between the number of the polymorphic CAG repeats (polyglutamine region) or GGN repeats (polyglycine region) in the androgen receptor gene with prognosis and also with...
the risk of developing prostate cancer (7, 12, 15, 18, 21). There may also be interaction between the CAG and GGN repeats. Stanford et al. reported that, when the subgroup in which both alleles were short (CAG <22 and GGN ≤16) was compared with the subgroup in which the alleles were long (CAG ≥22 and GGN >16), an odds ratio of 2.05 was observed (18).

In our study, PC-EW cells had a CAG repeat length of 19 and PC-OR a CAG repeat length of 22, compared with a normal length of 23 in US Caucasians. The shorter lengths of CAG repeats in the PC-EW cell line is compatible with the positive association of repeat length and the risk of prostate cancer described by several groups (13, 17, 18, 20, 21), but not with the longer repeat lengths in the PC-OR cell line.

Compared to the normal length of 23 repeats in US Caucasians, the polyglycine repeat area in PC-OR shows a normal GGN repeat length of 23. In contrast, in PC-EW cells, the GGN repeat length of 17 is significantly shorter than in normal Caucasians.

To sum up, in contrast to the androgen-sensitive PC-EW cell line, the PC-OR cell line shows a normal length of GGN and CAG repeats.

**Conclusion**

Point mutations of hAR are not necessary for every metastatic prostate cancer. Alterations in the polyglutamine and polyglycine repeat region in exon 1 of the hAR gene are often found. The PC-EW cell line shows an alteration with shorter lengths of CAG and GGN repeats. The other metastatic tissue cell line PC-OR shows normal lengths in both regions. Interestingly, we observed a slight heterogeneity in the GGN repeat length in both cell lines. It seems that alterations in the repeat regions in exon 1 are not necessary for the metastatic phase of prostate cancer. These repeats are two of many genetic changes that contribute to the overall risk for developing prostate cancer.
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References


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