Abstract. 
Background: Prostate cancer is the second leading cause of death among men in Western countries. Genetic alterations of the estrogen receptor gene are known to be indicative of a higher risk of this disease. The estrogen receptor gene is found as two subtypes, α and β. In this study the estrogen receptor α and β genes were tested in 2 human prostate cancer cell lines: the hormone-sensitive PC-EW and the hormone-independent PC-OR. Materials and Methods: Genomic DNA was isolated from 2 cell lines from metastatic prostate adenocarcinoma in hetero-transplanted male athymic nude (nu/nu) Balb/c mice. Mutation screening was performed by sequencing of exons 1-8 and intron 1 of the human estrogen receptor gene α, and exons 1-9 of estrogen receptor gene β. Results: No point mutations were detected in the ER gene subtypes of either cell line. Polymorphisms were found of ER-α in exon 1, intron 1, exon 3, 4, 5, intron 6 and exon 8 and of ER-β in intron 2 and exon 9. Conclusion: Point mutations of ER-α and -β are not necessary for metastatic prostate cancer, alterations in different areas of the ER genes are more often found. These polymorphisms are a part of many genetic influences that accumulate to contribute to men's overall risk for developing prostate cancer.

Prostate cancer is the most frequently diagnosed malignancy and the second leading cause of death from cancer among men in Western countries (1, 2). Estrogens have been shown to be involved in carcinogenesis and tumor progression in vitro and in vivo (3-5).

Several studies indicated the association of estrogen receptor gene polymorphisms with high-grade tumors, risk of progression and advanced disease in prostate cancer (6-8). The estrogen receptor gene is found as two subtypes, α and β (9). The ER-α gene is greater than 140 kilobases, contains eight exons and is located on chromosome 6q25.1 (10, 11). The ER-β gene is greater than 40 kilobases, contains nine exons and is located on chromosome 14q22-q24 (12).

Elucidation of the role of ER-α and -β gene mutations in the carcinogenesis of prostate cancer was the aim of our study. Mutation screening tests were performed in 2 human adenocarcinoma cell lines of the prostate with the question whether ER-α and -β gene mutations or polymorphisms are found or not. The androgen-sensitive PC-EW and the androgen-independent PC-OR prostate cancer cell lines were from lymph node metastases from two human pT3, N2, M0 prostatic adenocarcinomas.

Materials and Methods

The origin of the PC-EW and PC-OR tumor tissue and their establishment and maintenance by serial transplantation in nude mice is well documented (13-15). New oligonucleotide primers were designed encompassing the coding exons 1-8, intron 1 and the GGGA-repeat of the ER-α gene and the coding exon 1-9 of the ER-β gene (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 1 unit of Taq DNA polymerase (Invitrogen, Karlsruhe, Germany). Additionally, 1 M betaine was added for the amplification of exon 1, 3 and 4 of the ER-α gene and for the amplification of exon 1, 3, 4, 5, 6, 7, 8 and 9 of the ER-β gene. The PCR was performed with an initial denaturing step at 95°C for 3 min, then 33 cycles beginning with 95°C for 1 min, followed by an annealing step for 1 min (ER-α gene: 60°C for exon 1 and 4, 56°C for exon 2 and 8, 54°C for exon 3, 5, 6 and 7; ER-β gene: 60°C for exon 1-9), an elongation step for 1 min and 30 s 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).
No point mutations were detected in which the ER gene subtypes of both cell lines differed. However, we identified several single nucleotide polymorphisms (SNPs) in exon 1, intron 1, exon 3, 4, 5, intron 6 and exon 8 of ER-α, and in intron 2 and exon 9 of ER-β (Table II and III).

Table I. Oligonucleotide primers of ER-α gene exon 1-8, intron 1 and ER-β gene exon 1-9.

Table II. The following polymorphisms were identified in the ER-α gene.

Table III. The following polymorphisms were identified in the ER-β gene.

**Discussion**

Estrogens have been established as being essential for normal development of the prostate gland (16). However, in vitro and in vivo studies show estrogens to play an important role in prostatic carcinogenesis and to have an effect on growth in prostate cancer cell lines (3-5, 17). The genetic basis for this carcinogenic effect of estrogens is not well understood. Mutations of the ER-α and -β gene are not well documented in prostate carcinoma. Several studies (prostate cancer patients compared to healthy controls) indicate the association of ER-α receptor gene polymorphisms with an increased risk of developing prostate carcinoma, high-grade tumors, risk of progression and advanced disease (6-8).

In our study, we found these polymorphisms in the ligand-independent transactivation domain (exon 1/intron 1) and in the ligand-binding domain (exon 4) of the ER-α gene. Interestingly, the susceptible T/T genotype of the PvuII polymorphism in intron 1 was found in both cell lines, and the susceptible genotypes C/C in exon 1 and G/G in exon 4 was found only in the hormone-sensitive EW-cell line.

For the XbaI polymorphism in intron 1 of the ER-α gene, we identified the A/A genotype in both cell lines. No association with prostate cancer was observed (7, 18). For the GGGA-repeat, we identified five repeats (5/5 genotype) in ANTCANCER RESEARCH 27: 2071-2074 (2007)
both cell lines. Variants of this polymorphism may be associated with an increased risk of developing familial prostate cancer, as shown in Caucasian men (19). Neither genotypes of either cell line in exon 3 and exon 8 of the ER-α gene are associated with prostate cancer (6, 19).

In exon 5, we found an unknown common point mutation of both cell lines, with a subsequent amino acid change V400G. Interestingly, a Val substitution for Gly at amino acid 400 (G400V) in the ligand-binding domain with a lower hormone-binding capacity in vitro has been described (20, 21). Further studies are needed for an identification of this gene area as a new risk factor for prostate cancer.

In intron 6 of the ER-α gene, we found the G/G genotype in the EW cell line and the T/T genotype in the OR cell line. This polymorphism in the ligand-binding domain has not been reported together with prostate cancer until now. Interestingly, we found the T/T genotype variant in the hormone-independent OR cell line. This polymorphism may play a role in androgen sensitivity or independence.

In the ER-β gene, we found 2 polymorphisms, these have not formerly been described together with prostate cancer. Heterozygosity in the noncoding region (intron 2) was found in both cell lines. In 3’UTR of exon 9, which, together with exon 4-8, encodes for the ligand-binding domain, we identified different genotypes in both cell lines: the G/A genotype in the EW cell line and the A/A genotype in the OR cell line. Another G/G genotype was previously described in context with a susceptible haplotype in ER-β gene in a breast cancer disease association study (22).

Although polymorphisms with different genotypes in both cell lines are found in the noncoding region in intron 6 of the ER-α gene and in the 3’UTR of exon 9 of the ER-β gene, gene expression could be affected via structural change in RNA, leading to an alteration in processing or efficiency of translation. Further investigations are needed.

Conclusion

Mutations of the ER-α and -β gene are not necessary for hormone independence; alterations in the ligand-independent transactivation domain (exon 1), DNA-binding domain (exon 3) and the ligand-binding domain (exon 4-8 and 9) are more frequent. These identified alterations in exon 1, 4, 5 and 9 and in introns 1, 2 and 6 of the ER-α and -β gene may be some of many genetic alterations that accumulate to contribute to men’s overall risk for developing prostate cancer.

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References


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