Polymorphisms in the Human Progesterone Receptor (PGR) Gene of Two Human Prostate Adenocarcinoma Cell Lines

DIRK G. ENGEHAUSEN1, SABINE ENDELE2, STEFFEN F. KRAUSE1, TORSTEN RITH1, KARL M. SCHROTT1 and ZIYA AKCETIN1

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

Abstract. Background: The incidence and mortality rate of prostate cancer has been steadily increasing in most countries worldwide. A potential implication of the progesterone receptor has been reported in prostatic carcinogenesis. In this study, an allele of human progesterone receptor gene (PROGINS), which was demonstrated to be associated with an increased risk of sporadic ovarian cancer, was tested in two human prostate cancer cell lines, PC-EW and PC-OR. Materials and Methods: Genomic DNA was isolated from the cell lines in athymic nude mice. The polymorphisms in exon 4 and exon 5 and the insertion in intron G (PROGINS) were identified by sequencing and gel electrophoresis. Results: The PROGINS allele and the polymorphisms in exon 4 and exon 5 were found heterozygous in the PC-OR cell line but not in the PC-EW cell line. Conclusion: We described the polymorphisms of exon 4, exon 5 and PROGINS in prostate cancer. Mutation screening of the PGR gene may provide information for risk assessment of developing prostate cancer.

Over the past three decades, the incidence and mortality rate of prostate cancer has been steadily increasing in most countries worldwide. Treatment of the metastatic disease is an important goals. A potential implication of a member of the steroid hormone receptor family, the human progesterone (hPR), has been reported in prostatic carcinogenesis. This PGR gene is located at chromosome 11q22-q23, spans over 90 kilobases (kb) and contains eight exons. The hPR represses estrogen receptor gene activation (1). A 306-bp Alu sequence insertion in intron G of the PGR gene has been described (2). This insertion, called PROGINS, is combined with a silent mutation in exon 5 (codon 770) and a mutation in exon 4 (codon 660), causing an amino acid change from valine to leucine in the hinge region of the hPR. Studies have shown that there is an association between this PROGINS polymorphism and ovarian or breast cancer risk in different ethnic patient populations (3-5). Some of these studies were of borderline statistical significance. The PROGINS polymorphism of PGR is also increased in female offspring with maternal exposure to diethylstilbestrol (6). Genetic alterations of the progesterone receptor gene in prostate cancer have not been described until now. In this study, an allele of human progesterone receptor gene (PROGINS), which was demonstrated to be associated with an increased risk of sporadic ovarian cancer, was tested in 2 human prostate cancer cell lines, PC-EW and PC-OR. Both tumor cell lines were from metastatic tissues of advanced human tumors.

Materials and Methods

High molecular weight genomic DNA was extracted from 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 origin of the PC-EW tumor tissue and the establishment and maintenance by serial transplantation in nude mice have been previously described (7-9). 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. Histopathology showed a poorly-differentiated tumor (G3). PC-OR is an androgen-independent carcinoma, first described in 2001 (10). None of the carcinomas were treated with chemotherapy prior to surgery or collection of the cells for heterotransplantation.
Mutation screening was performed by sequencing and gel electrophoresis, applying the same primers and PCR conditions as in our previous studies (2, 4, 6), except the primers of exon 4 (Exon 4-hPR-Sense 5’- GTCAGAGTTGTGAGAGCACTGGATG -3’ and Exon 4-hPR-Antisense 5’- CTGGCAATGATTTAGACCATCTTGATG -3’).

Results

The PROGINS polymorphism was heterozygous in the PC-OR cancer cell line combined with the previously described mutations in exon 4 (codon 660, an amino acid change from valine to leucine) and in exon 5 (a silent mutation in codon 770). In the PC-EW cell line, no point mutations in exons 4 and 5 and no PROGINS polymorphism in intron G were detected (Figure 1).

Discussion

Genetic alterations of a steroid hormone receptor gene, the human androgen receptor (hAR) gene, are known in the advanced metastatic prostate cancer and also in the androgen-independent disease (11). Genetic alterations of the progesterone receptor gene have not yet been described in prostate cancer. A genetic mutation of the PGR gene...
changes the receptor function and, via feedback, the estrogen regulation. A polymorphism called PROGINS is known in different gynecological tumors. An association between the PROGINS polymorphism and ovarian or breast cancer risk in different ethnic patient populations was of borderline statistical significance.

Latil et al. (2001) reported no significant association between PGR expression and prostate tissues, using 23 tumors of different stages and 4 normal prostate tissues. The PGR displayed a wide range of expression in hormone refractory tumors: 4 tumors showed a decreased expression of PGR and 5 showed an increased expression. These differences were treatment-independent: up- and down-regulation were observed both in patients treated with antiandrogens or otherwise treated with LHRH agonists. The PGR is an estrogen-regulated gene with a significant positive association with the expression of hERα and hERβ, both subtypes of the hER (human estrogen receptor gene) (12).

The dimerisation of the mutated hPR in the PROGINS polymorphism is more stable than in a normal hPR (5). The heterozygous situation for PROGINS polymorphism in the androgen-independent prostate cancer cell line PC-OR suggests a change in the receptor function and, via feedback, in the estrogen regulation. It is still unknown whether these mutation changes in PC-OR play a role in androgen sensitivity or independence in prostate cancer.

**Conclusion**

We first describe the polymorphisms of the PGR gene in the human PC-OR prostate cancer cell line. We found the PROGINS polymorphism (a 306-bp Alu-insertion in intron G) combined with an amino acid change from valine to leucine in exon 4 (codon 660) and a silent mutation without amino acid change in exon 5 (codon 770). No point mutations of exon 4 (codon 660), exon 5 and in intron G were detected in the PC-EW cell line. The difference of these polymorphisms between the androgen-sensitive PC-EW cell line and the androgen-independent PC-OR cell line may influence the PGR gene and the hPR protein in hormone-sensitive and refractory tumors. The PROGINS allele may be one of many genetic influences that cumulate to contribute to overall risk for developing prostate cancer.

**Acknowledgements**

This study was supported in part by institutional grants of the Department of Urology and the Institute of Human Genetics of the University of Erlangen-Nuremberg, Germany.

**References**


Received August 2, 2004
Accepted January 31, 2005