Estrogen receptor β transcript variants associate with oncogene expression in endometrial cancer

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Abstract. The human ESR2 gene codes for estrogen receptor β1 and for multiple splice variants, which are suggested to exert distinct functions in the cellular estrogen response. Given that the function of ERβ in endometrial cancer remains unclear, we examined the expression of ERβ1, ERβ2 and various further ERβ transcript variants and their association with selected cancer-related genes in 74 human endometrium samples and endometrial cancer specimens by means of RT-qPCR. Additionally, we knocked down ERβ expression in HEC-1A endometrial adenocarcinoma cells by means of siRNA transfection. Expression of four ERβ transcript variants was significantly elevated in cancer tissue or in G3 tumors compared to postmenopausal endometrium. Expression of ERβ1, ERβ2, ERβ5 and five further variants was associated with the oncogenes MYBL2 or HER2 in endometrial cancer. In addition, siRNA-triggered knockdown of ERβ expression led to a significant decline of MYBL2 mRNA and protein levels in endometrial cancer cells. Our observation of increased ERβ transcript levels in cancer tissue and particularly their correlation with the expression of oncogenes, as well as the results of our knockdown studies, suggest a role of ERβ in endometrial carcinogenesis.

Introduction

Endometrial cancer is known to be a hormone-dependent neoplasia which is caused by a stepwise accumulation of molecular alterations in cellular pathways regarding proliferation and differentiation. Expression of steroid hormone receptors like estrogen receptors (ER) α and β and proges-
Patients and methods

Patients. In this study, we examined a total of 74 endometrial samples including 28 samples from normal endometrium and 46 cases of endometrioid endometrial carcinoma. The endometrial tissue specimens were collected between 2007 and 2009 by the Second Department of Gynecology of the Medical University of Lublin, Poland and by the Clinic of Obstetrics and Gynecology, Medical University of Regensburg, Germany. From the 11 premenopausal patients, aged 43-53 years, 5 were in the proliferative phase and 6 in the secretory phase of the menstrual cycle. The 17 postmenopausal women were aged between 46 and 90 years. Normal endometrial tissue was obtained from women subjected to surgery for reasons other than pathology of the endometrium, mainly cervical cancer. The group of endometrial cancer patients (endometrioid subtype), ranging from 54 to 82 years of age, underwent curative resection and could be divided into grading subgroups (G1, 15 cases; G2, 16 cases; G3, 15 cases). Informed consent for their participation in the studies was obtained from all patients. Tissue samples of patients were collected in accordance with German or Polish regulations and in agreement with the Ethics Committees of the University School of Medicine in Lublin, Poland or the Medical University of Regensburg, Germany. Complete clinical data were available for every patient. Immediately following surgery, tissues were stored in liquid nitrogen until RNA extraction.

Materials. DMEM/F12 culture medium was obtained from Invitrogen (Karlsruhe, Germany) and FCS was purchased from PAA (Pasching, Austria). HEC-1A endometrial adenocarcinoma cells were obtained from the American Type Culture Collection (Manassas, VA, USA). M-MLV-P reverse transcriptase was purchased from Promega (Madison, WI, USA). RNase-free DNase Set and the QuantiTect SYBR-Green PCR kit were obtained from Qiagen (Hilden, Germany). PCR primers were synthesized at the QuantiTect SYBR-Green PCR kit were obtained from Qiagen (Hilden, Germany). PCR primers were synthesized at the QuantiTect SYBR-Green PCR kit were obtained from Qiagen (Hilden, Germany). PCR primers were synthesized at the QuantiTect SYBR-Green PCR kit were obtained from Qiagen (Hilden, Germany). PCR primers were synthesized at the QuantiTect SYBR-Green PCR kit were obtained from Qiagen (Hilden, Germany). PCR primers were synthesized at the QuantiTect SYBR-Green PCR kit were obtained from Qiagen (Hilden, Germany). 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Antibodies and western blot analysis. HEC-1A endometrial adenocarcinoma cells were lysed in RIPA buffer [(1% (v/v) Igepal CA-630, 0.5% (w/v) sodium deoxycholate, 0.1% (w/v) sodium dodecyl sulphate (SDS) in phosphate-buffered solution (PBS) containing aprotonin and sodium orthovanadate]. Aliquots containing 15 µg of protein were resolved by 10% (w/v) SDS-polyacrylamide gel electrophoresis, followed by electrotransfer to a PVDF Hybond (Amersham, Buckinghamshire, UK) membrane. Immunodetection was carried out using monoclonal MYBL2 (M02) antibody (clone Table I. PCR primer sequences used for qPCR.

<table>
<thead>
<tr>
<th>Target/splice variant</th>
<th>Primer (5'→3')</th>
<th>Amplicon (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERβ1</td>
<td>GGCATGCGAGTAACAAGGGC GGGAGCCCTCTTTTGCTTTT</td>
<td>177</td>
</tr>
<tr>
<td>ERβ2</td>
<td>GTTTGGGTTGATTGCAAGAG TCTGCCCTCGCATG</td>
<td>101</td>
</tr>
<tr>
<td>ERβ5</td>
<td>GTTTGGGCTTGGCAAGAG CGTACCTCGCATGCTGA</td>
<td>146</td>
</tr>
<tr>
<td>ERβΔ1</td>
<td>TGGTTTCTGAAGAGAGACACTGA CTTCAACAGACCAGACTCTCA</td>
<td>151</td>
</tr>
<tr>
<td>ERβΔ2</td>
<td>ACCACAAGCCCAAATGTGTT ATCATATGTCCTGTATAGGTAAG</td>
<td>157</td>
</tr>
<tr>
<td>ERβΔ3</td>
<td>TCAAGGCTCCCCGGAGAGAG CAGGAAGGATGACGACTGC</td>
<td>172</td>
</tr>
<tr>
<td>ERβΔ4</td>
<td>TTGTCCACGCTCAAAATTCACTGT TCCACAAAGCCACACTTCAC</td>
<td>112</td>
</tr>
<tr>
<td>ERβΔ6</td>
<td>TTCTGGACAGTGATTGTAACCTCCTG GGGACAGGACATCAGGAG</td>
<td>175</td>
</tr>
<tr>
<td>ERβΔ1/2 (0K)</td>
<td>AATATGTTCTGAAGGACATAGA CTTACATCCCACCTCGT</td>
<td>128</td>
</tr>
<tr>
<td>ERβΔ1/2 (0N)</td>
<td>CTGCCTTTCTCAACAGGGTG CATTATGTCCTTGAGATAAAACAC</td>
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<tr>
<td>ERβΔ2/3</td>
<td>AAACAGGCTCCCAGGAGAGAGACACCTCACTGATGAG</td>
<td>145</td>
</tr>
<tr>
<td>ERβΔ3/4</td>
<td>AGCATTCAAGGCTTTGTGGA CCAGACAAAGATCTGGAGCA</td>
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<td>ERβΔ4/5</td>
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<tr>
<td>ERβΔ1/2/3 (0K)</td>
<td>CTGAAGGCTCCCAGGAGAGAG GCGCAATCCACCTGACAGA</td>
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<tr>
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<td>CTGCCTTTCTCAACAGGGTG CTTACAGCTGGAGATGAC</td>
<td>120</td>
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<tr>
<td>ERβΔ3/4/5</td>
<td>ATTCAGGAGATGGGGAAA GATCATGGGCTTGACAGA</td>
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<td>ERβΔ4/5/6</td>
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<td>ERα</td>
<td>CACATGAGTAACAGAGGGCTCG ATGAGGCTAGCCGGCAGTG</td>
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<td>PR</td>
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<td>130</td>
</tr>
<tr>
<td>HER2</td>
<td>CATGGTCAATGTGGAGATT CCTACATTCTGGGATGACCA</td>
<td>116</td>
</tr>
</tbody>
</table>
1C7, antikörper-online.de, Germany) or β-actin antibody (8226, Abcam, Germany) diluted 1:5,000 in PBS containing 5% skim milk (w/v) followed by horseradish peroxidase-conjugated secondary antibody which was detected using a chemiluminescence (ECL) system (Amersham).

Statistical analysis. Statistical analysis of the data was carried out using the InStat software (GraphPad Software). The statistical significance of the molecular gene expression changes in the endometrial carcinoma and the normal endometrium control group was assessed using the nonparametric Mann-Whitney test. Furthermore, we compared the gene expression of separate subgroups (G1, G2, G3) with the nonparametric Mann-Whitney test. The Spearman’s rank correlation was used to evaluate the correlation between the expressions of two target genes. P-values <0.05 were considered statistically significant.

Results

Differential gene expression in human endometrium and endometrial cancer. We analyzed expression of ERβ1, ERβ2, ERβ5, the further exon-skipped ERβ-isoforms, ERα, PR, and the cancer-related genes MYBL2, PTEN, Cyclin A2, Cyclin B1, Cyclin D1 and HER2 in a total of 74 endometrial tissue samples by means of RT-qPCR.

Transcript levels of ERβ1 and 2 did not differ between the cancer- and the control group (Fig. 1) or between the grading subgroups (data not shown). Expression of ERβ5 was about 3-fold elevated in endometrioid endometrial cancer, particularly in the G3 subgroup when compared to the postmenopausal endometrium (P<0.01) (Fig. 2). The exon-skipped splice variant ERβΔ1, transcribed from the 0N promoter of ESR2 gene, also was 3-fold elevated in endometrial cancer (P<0.01) (Fig. 1), particularly in the G1 and G2 subgroup.
Increased transcript levels in cancer tissue were also detected analyzing the exon-skipped isoform ERβΔ2/3 (P<0.05) (Fig. 1). In contrast, mRNA levels of the exon-deletion variant ERβΔ4 were significantly, about 3-fold decreased in endometrial cancer tissue (P<0.0001). Particularly the G2 and G3 subgroup exhibited a lower expression of this variant when compared to postmenopausal endometrium (both P<0.001). Expression of the other ERβ splice variants tested did not significantly differ between the compared groups or subgroups.

When we analyzed ERα transcript levels for comparison, we did not observe a difference between the cancer and the control group, but a significantly smaller expression was noted in the G3 subgroup compared to the normal endometrium (P<0.01) or the G1 (P<0.001) or G2 tumors (P<0.05).

Analysis of the ERβ/ERα ratio revealed a significant increase in G3 tumors when compared to the postmenopausal endometrium. This was true for the ratios ERβ1/ERα, ERβ2/ERα (both P<0.01) and particularly for the ERβ5/ERα ratio (P<0.001) (Fig. 3).

Our data also revealed differences in the expression patterns of cancer-related genes. Expression of the oncogene MYBL2 was about 4.5-fold higher in endometrial cancer than in pre- or postmenopausal endometrium (P<0.0001) (Fig. 4). We observed this significant increase of MYBL2 expression in all grading subgroups (P<0.0001). The cell cycle gene cyclin B1 exhibited a 3.9-fold elevated expression in endometrial cancer tissue compared to normal endometrium (P<0.001) (Fig. 4), particularly in G2 tumors (P<0.01) and G3 tumors (P<0.0001). Cyclin D1 was 4-fold elevated in the cancer group (P<0.001), whereas cyclin A2 mRNA levels were doubled in endometrial
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Figure 3. Ratio between expression of ERβ splice variants 1, 2 or 5 and ERα transcripts in postmenopausal endometrium and in G1, G2 and G3 graded endometrial carcinoma as assessed per means of RT-qPCR. *P<0.01 vs. postmenopausal endometrium; **P<0.001 vs. postmenopausal endometrium.

Association of ERβ1 and its splice variants with oncogenes and cancer related genes. To examine the potential significance of ERβ expression in endometrioid endometrial cancer, we first correlated the levels of ERβ1 and its variants with expression of ERα, PR and other cancer-related genes and oncogenes using the nonparametric Spearman test.

First, this test showed the expected strong positive correlation of ERα transcript levels with PR expression (rho=0.55, P<0.0001). Expression of ERβ1 and ERβ2 was strongly associated (rho=0.79) and also correlated with transcript levels of ERα (rho=0.38), but not PR. In contrast, ERβ5 expression was weakly associated with ERβ2 expression (rho=0.36), but not with ERα or ERβ1 (data not shown).

Transcript levels of ERβ1 and 2 were found to be associated with the expression of the HER2 oncogene at the mRNA level. Additionally, 5 exon-deleted ERβ variants were observed to positively correlate with HER2 transcript levels (Table II). The expression of ERβ5 mRNA and of five exon-skipped variants was associated with the transcript levels of the oncogene MYBL2 (Table II).

Expression of ERβΔ1, Δ3 and Δ2/3 variants was associated with cyclin D1 mRNA levels, whereas ERβΔ3, Δ2/3 and Δ1/2/3 (0K, transcribed from the 0K promoter of the ESR2 gene), positively correlated with cyclin B1 expression (P<0.01, data not shown).

Knockdown of ERβ expression in HEC-1A endometrial adenocarcinoma cells. To examine the functional significance of the observed correlation between ERβ and expression of oncogenes, we then knocked down expression of ERβ by means of transient siRNA transfection in the endometrial adenocarcinoma cell line HEC-1A. Three days after transfection with ERβ-specific siRNAs, a maximum suppression of total ERβ transcript levels by about 72% was observed. In these cells, we also observed downregulation of MYBL2 mRNA by about 60%. When we performed an additional western blot analysis of MYBL2 protein expression, we also observed downregulation of this gene in HEC-1A cells treated with ERβ siRNA (Fig. 5). In contrast, HER2 expression was not altered after knockdown of ERβ, at the mRNA or at the protein level (data not shown).

Discussion

Estrogens have been shown to play a significant role in endometrial physiology and tumorigenesis (9). Both ERs are expressed in normal endometrium, but levels of ERβ have been reported to be lower than those of ERα (10-12).
ERα is thought to primarily mediate the proliferative effect of estrogens both in cyclic endometrium and in pathogenesis of endometrial proliferative diseases, but the function of ERβ in this tissue is not quite clear (13). Particularly the role of ERβ, coded by the human ESR2 gene, in endometrial carcinogenesis remains nebulous. The ESR2 gene codes for the estrogen receptor β1 and for multiple splice variants resulting from alternative exon usage or exon skipping, which are able to exert different functions in the cellular estrogen response (1,5,14). Thus, to elucidate the whole picture of ERβ action in endometrial cancer, we decided to examine expression of all relevant ERβ transcript variants.

Estrogen receptor β previously has been suggested to act as a tumor suppressor in endocrine responsive tissues like the breast and prostate (14-16). This hypothesis primarily arose from the observed decline of ERβ expression in cancer tissue and from in vitro data demonstrating a growth-inhibitory action of this receptor (17-19). Whereas some studies supported the idea that ERβ might also act as a tumor-suppressor in the endometrium, primarily due to observation of decreased receptor levels in cancer tissue (20-22), other studies did not (22-26).

The results of our study do not suggest the presence of an antitumoral effect of ERβ1 or of its splice variants in human endometrium. Transcript levels of 18 ERβ variants tested were found to be unchanged or even elevated in endometroid endometrial carcinoma. From these ERβ variants, four were significantly overexpressed in endometrial cancer or in G3 tumors. Eight ERβ isoforms were positively correlated with the expression of the oncogenes MYBL2 or HER2 and six associated with the cyclins B1 or D1. Supporting the results of our correlation study, and establishing a direct functional connection between ERβ and MYBL2, knockdown of ERβ in HEC-1A endometrial adenocarcinoma cells by means of RNAi led to downregulation of MYBL2 in this cell line. Only one ERβ isoform, ERβΔ4, was decreased in cancer tissue. Though being limited by the fact that we had to perform this study at

Figure 4. Expression of the indicated genes in postmenopausal endometrium and in endometrial carcinoma. Data were assessed at the mRNA level by means of RT-qPCR. *P<0.05 and ***P<0.001 vs. postmenopausal endometrium.
the mRNA level because no specific antibodies existed for the 15 exon-deleted ERβ splice variant tested, the results of our study clearly suggest that ERβ and most of its splice variants do not act as tumor suppressors in human endometrium, but may have tumor-promoting properties.

ERβ1, 2 and 5 are known to be differentially expressed in hormone-dependent cancer (1-4). Whereas ERβ1 expression has been described to decline in breast and prostate cancer tissue, this effect seems not to be present in endometrial carcinoma (27-29). In line with previous studies, we did not observe altered ERβ1 transcript levels in endometrial cancer tissue (30,31). ERβ1 was also previously found not to be associated with the depth of myometrial invasion (31). With regard to ERβ2, some reports demonstrated a decline of this variant in endometrial cancer (31), whereas others found this receptor variant not to be altered in endometroid cancer tissue (30). Our results are in line with the latter report and these findings may be supported by the fact that, in contrast to ERβ5, transcript levels of ERβ2 were not associated with proliferation markers.

However, we found an unexpected, significant association of ERβ1, ERβ2 and 7 exon-skipped variants with HER2 receptor tyrosine kinase. Increased HER2 levels in breast and ovarian cancer as well as in different other malignancies have been reported previously (32) and are associated with accelerated tumor progression (32). Accordingly, we detected an overexpression of HER2 in endometrial cancer tissue compared to normal endometrial tissue. Given that expression of HER2 is associated with an aggressive tumor phenotype (33,34), its association with ERβ1, ERβ2 and 7 further variants might be another argument against a protective role of ERβ in endometrioid cancer (35). Our observation is in accordance with previous studies reporting association of ERβ1 with HER2 in breast cancer (36-38).

However, in other studies, expression of ERβ5 was reported to be unchanged or to be elevated in endometrial cancer tissue (30,39). Our study observing elevated ERβ5 mRNA levels in endometrial cancer, particularly in G3 tumors, is in line with the latter study and with reports from breast and prostate tissue (28,30,40). The demonstrated association of ERβ5 and 5 exon-skipped isoforms with the oncogene and cell cycle promoter MYBL2 (B-MYB) suggests that these ERβ variants may be involved in tumor growth. The results of our ERβ knockdown study employing the endometrial adenocarcinoma cell line HEC-1A not only corroborate these correlation data, but clearly suggest that MYBL2 transcript levels may be regulated by ERβ variants in this cell line. MYBL2 is a transcription factor of the MYB family, which is amplified and overexpressed in many tumor types like breast or prostate cancer (41,42). It is proposed that MYBL2 is involved in controlling cell proliferation and differentiation (43-46). Our data showing increased levels of the MYBL2 gene in premenopausal tissue support the relevance of this gene in endometrial proliferation.

Transcript levels of 6 exon-skipped ERβ variants were positively correlated with cyclin expression, again suggesting that ERβ isoforms are associated with proliferation of endometrial cancer. Upregulated expression of cyclin B1 (47), cyclin D1 (48-54) and cyclin A2 (55) in endometrial carcinomas as well as various other cancer types have been previously reported (56). Furthermore, cyclin B1, D1 and A2 have been found to be associated with high levels of Ki-67, indicating their implication in cell proliferation and progression of endometrial cancer (57-59).

Our data suggest a role of ERβ and its splice variants in endometrioid endometrial cancer. Whereas levels of ERβ1 and 2 were not altered in endometrial cancer tissue, the common ERβ variant 5 was overexpressed in endometrial cancer. The significant increase of four ERβ splice variants in cancer tissue and in G3 tumors and particularly the association of ERβ isoforms with the oncogenes MYBL2 or HER2 suggest that ERβ may be involved in the growth of endometrial cancer. These correlation data were corroborated by the results of our RNAi study establishing a direct link between ERβ and MYBL2 expression.

**Acknowledgements**

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**References**


9. Critchley HO, Henstra TA, Kelly RW, Scobie GS, Evans LR, Groome NP and Saunders PT: Wild-type estrogen receptor (ERβ) and the splice variant (ERβc*x/β2) are both expressed within the human endometrium throughout the normal menstrual cycle. J Clin Endocrinol Metab 87: 5265-5273, 2002.


