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TRANSCRIPTIONAL RATIO OF ESTROGEN RECEPTOR β mRNAs IN CARCINOMAS AND IN NORMAL TISSUES.

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The existence of two receptors for estrogen, ERα and ERβ, encoded by two different genes (ESR1 and ESR2), together with the existence of its isoforms and splice variants, imposes the need to clarify their function in estrogen signaling. In order to investigate if the weight ratio of estrogen receptor beta (ERβ1) and its splice variant (ERβΔ5) is different in malignant tissue compared to healthy tissue we analyzed their expression by the method of quantitative RT-PCR and showed that ratio ERβ1/ERβΔ5 in breast cancer and cell line MDA MB 361 is increased compared to healthy tissues. This finding suggests that decreasing of ERβΔ5 may be one of the phenomena related to tumorigenesis in estrogen responsive tissues and points to possible application of this type of analysis in future standard clinical practice

The status of estrogen receptor alpha (ERα) and that of progesterone receptor (PR) are routinely used in breast cancer treatment to select optimal therapy. Overall, approximately 60% of ERα-positive tumors respond to endocrine therapies. Although combination of ERα with PR level determination offers some improvement in the prediction of endocrine response, it still does not serve as an absolute predictor of responsiveness to endocrine therapy. Identification of the second estrogen receptor, named estrogen receptor beta (ERβ), as well as the existence of numerous isoforms/splice variants of both ERα and ERβ, suggests that complex regulation of estrogen function exists. After the cloning and characterization of ERβ, several isoforms and splice variants at the mRNA level were described but their biological significance has not yet been resolved. Deletion of one or more coding exons was among the first recognized mechanisms for generation of splice variants (V l a d u š i ć et al., 1998; L u et al., 1998; P o o l a et al., 2002). The splice variant without the fifth exon (ERβΔ5), which encodes truncated protein, was one of first recognized deletion variants of the ERβ transcripts, but little is known about its expression level in cancer. We propose that two mRNA transcripts of ERβ, viz., β1 encoding wild type receptor and Δ5, are differentially expressed in malignant and normal cells. In light of the fact that inhibitory activity of ERβΔ5 seems to be dose-dependent, we quantified the mRNA level of these two transcripts (ERβ1 and ERβΔ5) in normal and malignant tissues and in malignant cell lines. In this study, we analyzed the wight ratio isoform ERβ1 and ERβΔ5 mRNA. Here analyzed normal and breast carcinoma tissue (BC) and MDA MB 361 (a cell line from human breast adenocarcinoma), as well as normal testicular and uterine tissue. Expression of ERβ1 and ERβΔ5 was measured using sensitive and specific TaqMan probes by the method of quantitative RT-PCR.

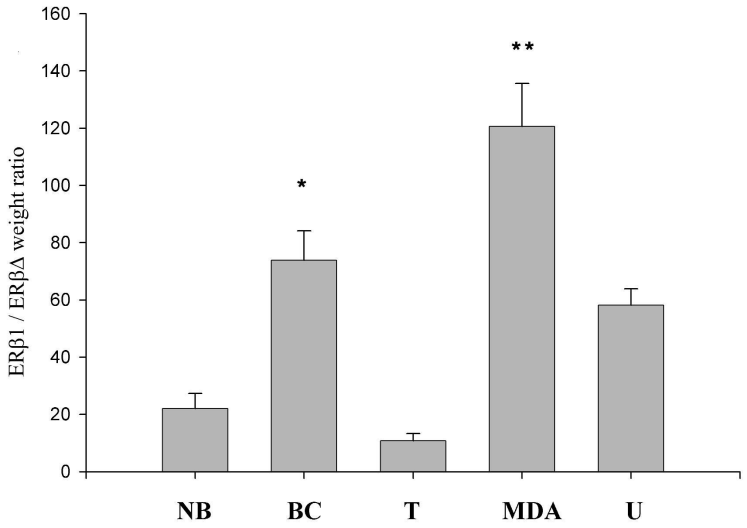
Analysis was perform on 34 samples obtained after surgery from patients with primary breast tumors hospitalized at the Institute of Oncology and Radiology of Serbia, Belgrade. These samples were chosen as positive for ERβ1 mRNA expression based on the cut-off value established in our previous study (M a n d u š i ć et al., 2006). Samples of adjacent normal tissues were obtained after total mastectomy. Samples of testicular tissue were obtained from the Institute of Pathology, Belgrade, while samples of uterus were obtained from patients who underwent hysterectomy at the Institute of Obstetrics and Gynecology in Belgrade. Tissue was stored on liquid nitrogen until RNA isolation. All patients met the following criterion: primary operable unilateral invasive breast carcinomas without previous treatment. The study was approved by the Institutional Review Board and adhered to the National Health Regulation Guidelines. Approximately 50 – 100 mg of tissue frozen in liquid nitrogen was pulverized in a cold mortar and extraction of total RNA was performed by the acid-phenol guanidine method according to C h o m c z y n s k i and S a c c i (1987). Quality and concentration of the RNA preparation were verified on 0,8% agarose gels stained with ethidium bromide. One μg of total RNA was reverse-transcribed over a period of 60 min at 37ºC in a reaction volume of 20μl with the Omniscript RT Kit (Qiagen, Hilden, Germany) using the 10-μM random hexamer and 1-μM oligodT(15) primer according to manufacturer instructions. All PCR reactions were performed using a 7000 Sequence Detection System (Applied Biosystems, Foster City,

CA). PCR was carried out in 25-µl reaction volume containing the 1x TaqMan Universal PCR Master Mix, 1x TaqMan Pre-Designed Gene Expression Assay (Applied Biosystems, Foster City, CA) and 10 µl of cDNA diluted with water (1:10). Assays were specific for target transcripts: assay ID Hs01100359_m1 for ERβ1 and assay ID Hs01105521_m1 for ERβΔ5. The ratio of examined transcripts ERβ1 and ERβΔ5 in each sample was expressed as N-fold differences relative to each other according to the equation: $N = 2\text{-}(Ct_{ER\beta1} - Ct_{ER\Delta5})$, where Ct is the threshold cycle. Differences in expression of ratio ERβ1/ERβΔ5 between healthy breast tissue and either BC or the MDA MB 361 cell line were analyzed by the Mann-Whitney and t- test, respectively.

The ratio of ERβ1 and ERβΔ5 mRNA was expressed as the N-fold difference in the relation to each other in examined samples. Expression of ratio ERβ1/ERβΔ5 in normal breast tissue is significantly lower than in BC ($p = 0{,}016$, Mann-Whitney, Fig. 1). In addition, expression of ratio ERβ1/ERβΔ5 in the adenocarcinoma cell line significantly differs from that in normal breast tissue ($p < 0{,}001$, t-test) and is similar to that found in BC. Normal uterine and testicular tissue as a control did not significantly differ from normal breast tissue regarding the ERβ1/ERβΔ5 ratio (Fig. 1).

These data suggest that an increased relative weight ratio of receptor ERβ1 and truncated receptor ERβΔ5 is connected with the process of malignant transformation. Increase of ratio ERβ1/ERβΔ5 in BC results mainly from more pronounced decrease of ERβΔ5 mRNA expression than of ERβ1 mRNA expression in breast tumors compared to normal breast tissue. We have shown (M a n d u š i ć et al., 2007) decrease of both ERβ1 and ERβΔ5 mRNA expression in breast tumors compared to normal breast tissue. Protein encoded by ERβΔ5 mRNA lacks the C terminal domain (responsible for ligand binding) but retains the domains for nuclear localization and heterodimerization. Such a receptor could, potentially, have an effect on transcriptional activities of both estrogen receptors, ERα and ERβ. It has been shown that ERβΔ5 protein in a cell transfection system attenuates E2-stimulated transactivation by ERβ1 and ERα in a dose-dependant manner (I n o u e et al., 2000). In contrast, ERβΔ5 alone lacks transcriptional activity even in the presence of a ligand (I n o u e et al., 2000). It follows that the ERβΔ5 receptor acts as an inhibitor of transcriptional activity of weight isoforms of ERα and ERβ. This means that decrease in inhibitory activity of ERβΔ5 during estrogen-stimulated proliferation may be involved in tumorigenesis. In conclusion, we point out the need to analyze the complete isoform profiles of ERβ, Erα, and PR in clinical samples, since it is possible that the pattern of isoform expression might be of prognostic and predictive value in clinics.

Fig. 1. Ratio of ERβ1/ERβΔ5 in normal tissue (NB - breast, T - testis, U – uterus), malignant tissue (BC - breast carcinomas), and an adenocarcinoma cell line (MDA mb 361). The Y axis represents relative expression ratio of two transcripts, ERβ1 and ERβΔ5, obtained from Ct values as described in the text.



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