Tamoxifen tailored treatment in breast cancer: splicing variants and pharmacogenetic profiles of ESR1 gene as new promising biomarkers

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Background Estrogen receptor positive breast cancer is the most frequent tumour in women. For its treatment and prevention the selective anti-estrogenic drug tamoxifen (TAM) is diffusely prescribed. TAM efficacy is fully recognized, but a significant percentage of patients do not benefit the therapy, limited by common intrinsic and acquired resistance. The nuclear estrogen receptor alpha (ERa), TAM molecular target, is synthesized from ESR1 gene, that is characterized by various common SNPs. ERa is activated by estrogen binding and acts regulating the expression of estrogen sensitive (Es) genes (e.g. MGP). Recently, tissue-specific ERa splice variants have been detected in different healthy and tumour tissues, co-expressed along with ERa complete form (ERa66). Among them the new unique splicing isoform ERa36 has been suggested as a novel biomarker of resistance in breast cancer.
The aim of our study was to investigate the action of TAM on ERa splice variants, in particular ERa36 isoform, and on the expression of Es genes, by using peripheral blood leukocytes as indicators of response to anti-estrogen treatment. In addition we would evaluate the role of several intronic SNPs in the ESR1 gene, potentially involved in ERa splicing events.
Methods A cohort of 34 women taking TAM as adjuvant therapy for early breast cancer was enrolled for this pilot study. A group of 100 aged-matched healthy women were used as reference control. Leukocytes from peripheral blood were collected to extract RNA. The ERa variants profiles have been characterized by Taqman probes, while the quantification of Es genes by SYBR-Green technology. The analysis of plasma concentrations of TAM and metabolites was conducted by an HPLC method. In the same group of patients 8 intronic SNPs have been determined by RFLP. A statistic regression analysis was applied.
Results ERa36 was the most expressed isoform, along the wild-type protein ERa66, in leukocytes, in a largely variable manner among patients. Moreover it presented an inverse correlation with the complete form ($p<0.0001$). In patients, MGP expression was significantly down-regulated compared to the control subjects ($p<0.0001$) but significantly correlated with ERa36 levels ($R=0.37$, $p=0.03$) suggesting that ERa36 isoform, that is unable to bind TAM, induced the transcription of MGP gene, when ERa66 was inhibited by the drug. In addition, in patients taking TAM, we observed significant reduced levels of ERa exon 5 deleted (Δ5) variant ($p=0.01$); on the contrary the isoform of ERa missing exon 7 (Δ7) was doubled in patients compared to controls ($p=0.06$).
According to their gene position and structure, two intronic SNPs, located at intron 5 (1236-112 A>G, *rs9322354*) and intron 6 (1369+123 G>A, *rs2207396*) were analysed in relation to the most expressed ERa variants (ERa36, ERaΔ5, ERaΔ7). We found that the expression of ERa36 and of ERaΔ7 was not correlated with the two intronic SNPs. On the contrary ERaΔ5 isoform was significantly down-regulated about 1.8 times ($p=0.03$) in those individuals who carried the intronic variation *rs9322354*. The SNP *rs2207396* (at intron 6) didn't revealed any influence on the expression of the exon 5 deleted variant.
Conclusions Leukocytes may be a valid tool to study the expression profiles of ERa variants and sensitive genes, in patients taking TAM. TAM significantly down-regulated the MGP gene and modulated the expression of 5- and 7-exon deleted variants. In addition a role of ERa36 isoform in treatment resistance may be surveyed. The significant correlation between the intronic SNP at intron 5 and the expression of the ERaΔ5 isoform suggest that a simple genotyping method could be a promising alternative approach to investigate the expression profiles of ERa splicing isoforms in each and every patient. Future investigations will help us to clarify the relation between ERa splicing variants and clinical outcome in breast cancer patients.

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