Evaluation of the role of the estrogen receptor splicing isoforms in the pharmacological response to endoxifen treatment in breast cancer models

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Background Breast cancer has the highest incidence in women and represents the leading cause of cancer-mortality in Western countries. Estrogen receptor positive breast cancer subtypes are the most frequent (60-80%) and for their treatment the selective estrogen receptor modulator tamoxifen (TAM) can be used. TAM efficacy is widely recognized in the adjuvant and advanced setting, but a significant percentage of patients do not benefit the treatment. TAM is a pro-drug extensively metabolized by the hepatic cytochrome P450 CYP2D6 into more active and powerful metabolites compared to the parental drug. Among these it has been recently taken into special account the active metabolite endoxifen (END), which is considered the main responsible for the therapeutic response because of its high affinity for the molecular target, the estrogen receptor alpha (Erα), and because it shows higher plasma levels compared to other active metabolites. Moreover, it has been widely reported that in tumor and healthy mammary tissues estrogen receptor splicing isoforms of Erα and Erβ are coexpressed with the full-length proteins and it has been suggested that they may interfere with estrogen pathways and END activity. The aim of our study was to investigate the role of two estrogen receptor isoforms Erα-Δ7 and Erβcx in the pharmacological response to the active metabolite END. **Methods** Two different in vitro models were used, MCF7-Erα-Δ7 and MCF7-βcx, derived from the stable transfection of a breast cancer cell line, wild-type MCF7, with the splicing variant of Erα missing exon 7 and the splicing variant of Erβ lacking exon 8 with 28 exclusive aminoacids, respectively. The MCF7-Erα-Δ7 strain was generated by our group, the MCF7-βcx cell line was kindly provided by Karolinska Institutet. Both strains were checked and confirmed for concomitant expression of the transfected isoforms with wild-type Erα protein by Western Blotting. Erα-Δ7 isoform trans-activity via EREs (Estrogen Responsive Elements) was characterized by luciferase assay. Before any molecular evaluation cells were kept under appropriate selection conditions, by antibiotics blasticidin (2μg/ml) and G418 (200μg/ml), respectively for MCF7-βcx and MCF7-Erα-Δ7, for at least 10 days. The gene expression levels of two known Erα target genes ADORA1 and IL20, implicated in the tumor progression, were monitored in the two transfected cell lines following estrogen (1nM) and END (20 and 40 nM) exposure for 24 hours by RT-qPCR, compared to MCF7 wild-type and only vehicle addition. **Results** Erα-Δ7 isoform showed no direct influence on ERE regulated gene expression evaluated by luciferase assay. IL20 and ADORA1 expression levels were significantly increased by estrogen exposure in transfected and wild-type cells (mean fold changes versus vehicle=+9.3±3.8 and +5.9±05, respectively); while ADORA1 increased similarly in the three lines, IL20 showed lower levels (about 50%) in MCF7-Erα-Δ7 after estrogen stimulation. The effects of END treatment were dose and gene dependent. In parental MCF7 estrogen+END (40nM) reduced the activation of ADORA1 and IL20 of 3.6 and 8.5 folds ($p<0.001$), respectively. IL20 gene expression was significantly reduced by END both in MCF7-Erα-Δ7 and MCF7-βcx by -5.0 and -7.5 ($p=0.007$) folds, respectively. On the contrary, END treatment reduced ADORA1 expression in MCF7-Erα-Δ7 (-4.6) but produced no change in MCF7-βcx mRNA levels. In addition, comparing the two transfected strains, END induced significantly lower ADORA1 ($p<0.0001$) and IL20 ($p=0.009$) expression levels in cells expressing the Erα-Δ7 isoform, suggesting a possible dominant-negative effect on Erα activity. **Conclusions** Erα-Δ7 and ER-βcx isoforms were shown to differently influence ERα trans-activity induced by estrogen and counteracted by END. If confirmed, these findings could help explaining differential and unpredictable outcomes in estrogen receptor positive breast cancer patients and personalizing TAM treatment.