Prognostic significance of estrogen receptor beta in early stage breast cancer

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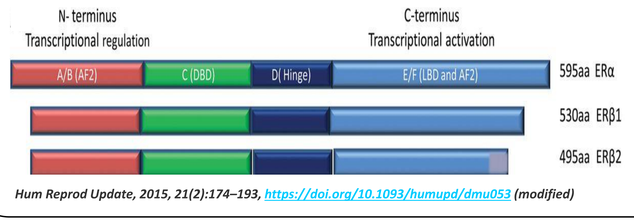
Introduction

Breast cancer (BC) is the most common cancer among women worldwide. Estrogen receptor (ERα) is a key player in breast cancer prognosis and treatment. Adjuvant endocrine therapy (AET) with antiestrogens or aromatase inhibitors is the treatment of choice for early-stage ERα-positive BC. ERα is engaged in extensive cross-talk with ERβ, the second ER isotype. There are five isoforms of ERβ (ERβ1–5), of which only ERβ1 has estrogen and antiestrogen binding ability.

ERβ1 and ERβ2 are the isoforms most frequently expressed in BC. The role of ERβ isoforms on breast cancer prognosis and treatment remains elusive.

Comparison of the domain structures of ERα and ERβ



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We have previously shown that while low ERβ1 expression is a marker of early relapse of early-stage ERα-positive BC following AET, high ERβ2 expression is a marker of late relapse *[Cancer Lett 2015;358:37-42. doi: 10.1016/j.canlet.2014.12.022]*.

Aim: to study the role of ERβ1 and ERβ2 expression in modulating the response of ERα-positive MCF7 cells to antiestrogens.

Experimental

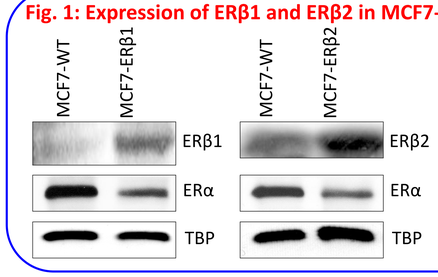
Cell lines:

MCF7-WT, a model of early-stage ERα-positive BC inherently expressing ERα
MCF7-ERβ1, a clone of MCF7 cells expressing human ERβ1
MCF7-ERβ2, a clone of MCF7 cells expressing human ERβ2

Ligands:

E2, estradiol (estrogen)
OHT, hydroxytamoxifen (antiestrogen, Novaldex)
ICI, ICI182,780 (antiestrogen, Faslodex)
DPN, diarylpropionitrile (ERβ-selective agonist)
MPP, methyl-piperidino-pyrazole (ERα-selective antagonist)
atRA, all-trans retinoic acid [Retinoic Acid Receptor (RAR) agonist]

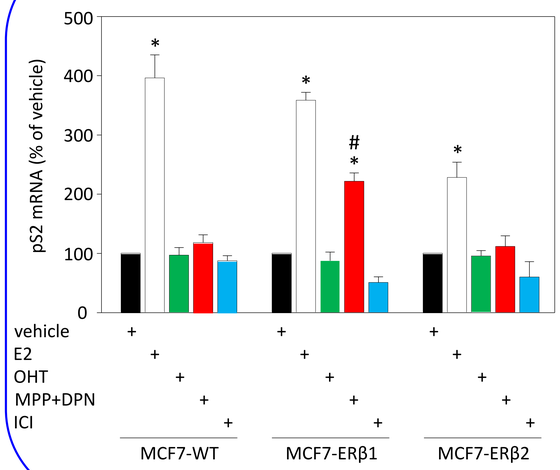
Fig. 1: Expression of ERβ1 and ERβ2 in MCF7-ERβ1 and MCF7-ERβ2 cells, respectively



Western blot analysis of nuclear fraction of MCF7-WT, MCF7-ERβ1 and MCF7-ERβ2 cells treated with 3 nM estradiol (E2).

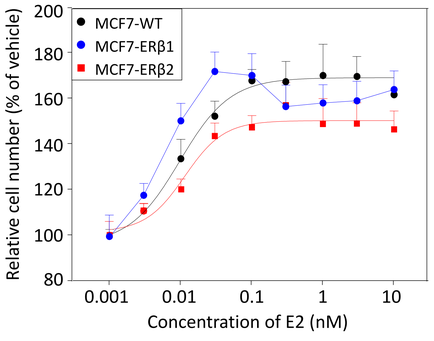
Expression of ERβ1 and ERβ2 results in lower levels of ERα expression in MCF7 cells .

Fig. 2: ERβ1 is transcriptionally active in the absence of ERα activation



MCF7-WT, MCF7-ERβ1 and MCF7-ERβ2 cells, cultured in steroid-free medium were treated with vehicle (0.1% DMSO), 10 nM E2 or 100 nM of each of OHT, MPP, DPN and ICI182,780. pS2 mRNA levels were assessed by RT-qPCR and expressed relative to the respective levels upon treatment with vehicle alone. Values are mean ± SEM of at least three independent experiments; *p<0.05 vs vehicle; #p<0.05 vs similarly treated WT cells.

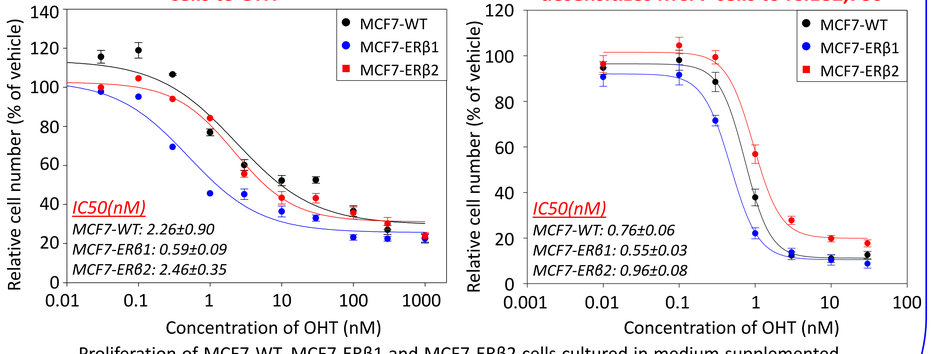
Fig. 3A: ERβ1 expression sensitizes MCF7 cells to low concentrations of estradiol



Proliferation of MCF7-WT, MCF7-ERβ1 and MCF7-ERβ2 cells cultured in steroid-free medium and exposed to vehicle or increasing concentrations of E2 for 72 h. Relative cell numbers were expressed as the percentage of that of vehicle-treated cells. Values are mean ± SEM of at least three independent experiments. DCC-FBS, dextran-coated charcoal-treated FBS.

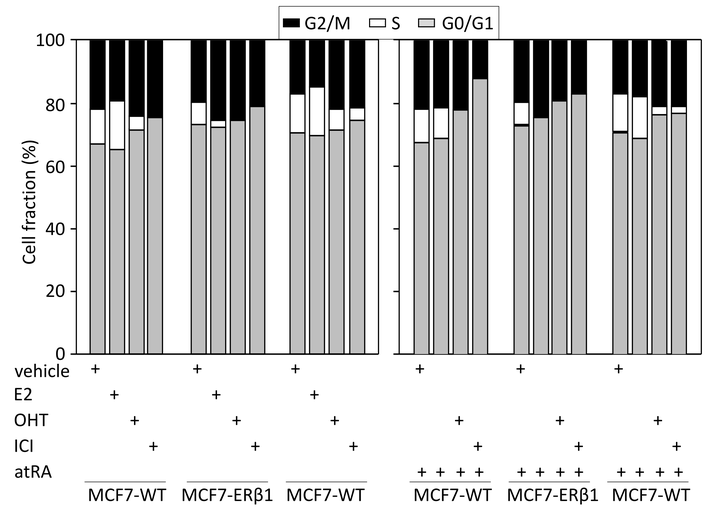
Fig. 3B: ERβ1 expression sensitizes MCF7 cells to OHT

Fig. 3C: ERβ1 expression sensitizes and ERβ2 desensitizes MCF7 cells to ICI182,780



Proliferation of MCF7-WT, MCF7-ERβ1 and MCF7-ERβ2 cells cultured in medium supplemented with 0.1 nM E2 for 24 h and subsequently treated with vehicle or increasing concentrations of OHT (left panel) or ICI182,780 (right panel) for 6 days. Relative cell numbers were expressed as the percentage of that of vehicle-treated cells. Representative graph of at least three independent experiments.

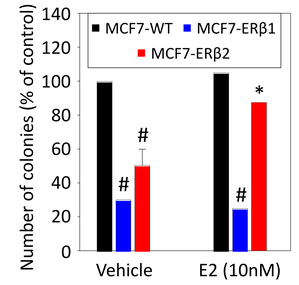
Fig. 4: ERβ1 sensitizes and ERβ2 desensitizes MCF7 cells to OHT and atRA



Cell cycle phase distribution of MCF7-WT, MCF7-ERβ1 and MCF7-ERβ2 cells cultured in medium supplemented with 0.1 nM E2, were treated with vehicle (0.1% DMSO), E2 (10 nM), OHT (100 nM), ICI182,780 (100 nM) or atRA (50nM), as indicated, for 72 h. Values are mean ± SEM of at least three independent experiments.

E2/OHT/ICI suppressed/blocked the G1>S transition of MCF7-ERβ1 cells , but failed to affect the G1>S transition of MCF7-ERβ2 cells .
atRA alone blocked G1>S transition of MCF7-ERβ1 cells, but failed to affect the G1>S transition of MCF7-ERβ2 even in combination with OHT or ICI.

Fig. 5: ERβ1 expression reduces anchorage-independence of MCF7 cells



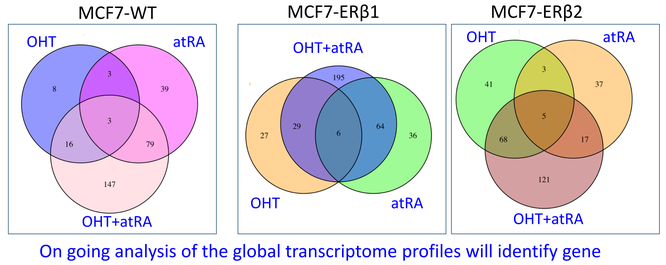
Colony formation for MCF7-WT, MCF7-ERβ1 and MCF7-ERβ2 cells cultured for 15 days in soft agar in the presence of vehicle (0.1% DMSO) or E2 (10 nM). The observed number of colonies was expressed as the percentage of the number of colonies from MCF7-WT cells in the presence of vehicle (control, set equal to 100). *p<0.05 vs same cell line in the presence of vehicle; #p<0.05 vs similarly treated MCF7-WT cells.

Predominantly decreased colony formation of ERβ1-expressing cells.

Fig. 6: Global transcriptome analysis of MCF7-WT, MCF7-ERβ1 and MCF7-ERβ2 cells

Differentially expressed genes	OHT (0.1 μM)	atRA (50 nM)	OHT+atRA (0.1 μM+50 nM)
MCF7-WT	30	124	245
MCF7-ERβ1	62	106	294
MCF7-ERβ2	117	62	211

Microarray analysis using mRNA form MCF7-WT, MCF7-ERβ1 and MCF7-ERβ2 cells, treated as indicated. Genes with an FDR<0.05 and a fold change >1.5 were considered as significantly differentially expressed genes.



On going analysis of the global transcriptome profiles will identify gene signatures associated with ERβ1-mediated sensitivity and ERβ2-mediated resistance of MCF7 cells to antiestrogens, retinoids and their combination.

Conclusions

- ERβ1 can be transcriptionally activated independently of ERα activation
- ERβ1 expression sensitizes MCF7 cells to OHT and to ICI182,780
- ERβ2 expression desensitizes MCF7 cells to ICI182,780
- ERβ1 sensitizes and ERβ2 desensitizes MCF7 cells to atRA
- ERβ1 expression reduces anchorage-independence of MCF7 cells

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