Significant ERβ Isoform Expression in ERα-negative and Triple Negative Breast Cancers

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BACKGROUND

- ERα has been the main accepted prognostic and predictive marker of breast cancers (BC) to SERM, however, approximately 50% of ERα+ BC are de novo resistant to SERM or acquire tamoxifen (TAM) resistance despite expression of ERα. On the other hand, about 5% to 10% of patients with ERα-negative tumors do respond to TAM and the responsible factors are not clear.
- ERβ, the second ERβ isotype has been recently identified. ERα and ERβ are distinctly different in genotype, tissue distribution, transcriptional activities in certain ligand, interaction with gene regulatory sites and binding pharmacological agents. ERβ has at least five isoforms (ERβ1-5) and splice variants.
- ERβ is co-expressed with ERα and has shown to modulate ERα activity. The ratio of ERα:ERß has been shown to predict progression to neoplasia and malignancy in BC.
- ERβ is expressed in both normal and neoplastic human breast tissue and is frequently expressed in stromal cells and in the cytoplasm/membrane.
- Most previous clinical studies of ERβ were done in ERα+ BC and the results have been contradictory: some showed ERβ with favorable outcome to TAM treatment and tumor suppressive, and others with poor prognosis. Recent gene expression profiling studies have shown that ERβ probably regulates a distinct subset of genes involved in cellular proliferation and apoptosis and is anti-proliferative.
- We and few others have demonstrated a significant ERβ expression in aggressive ERα negative BC. ERβ may become the potential new therapeutic target in this sub-cohort.

AIMS

To evaluate the significance of ERβ isoform mRNA expression in ERα negative and triple negative(TN) BC, by correlating ERβ isoform expression with a large number of biomarkers and clinical and pathological parameters in this sub-cohort of BC.

MATERIALS & METHODS

Study subjects: 143 BC (69 cases of ERα negative including 43 TN and 72 ERα positive) obtained over the period from 2003 to 2010 were from the patients with ages of 40 to 88 years, with clinical F/U ranging from 13 to 132 months. They were treated with radiation, hormone or chemotherapy (CHT) or their combination. Only 2 TN were treated with TAM.

Materials: The tumor sizes ranged from 0.5 cm to over 5 cm; the histologic types were: 114 IDC, NOS, 7 atypical medullary, 6 medullary, 3 apocrine, 3 lobular, 2 inflammatory, 5 mixed, and 3 micropapillary. The tumor grades were: 78 grades 3, 52 grade 2, and 13 grade. The tumor stages were: 73 stage 1, 39 stage 2, 10 stage 3 and 10 stage 4.

Methods:

- mRNA of ERβ and ERα was analyzed by RT-qPCR from 2 cores of FFPES. Multiple breast cancer cell lines were tested together with FFPES to validate the quality of mRNA in FFPES. ERβ mRNA expression was concordant between breast cancer cell lines and human cancer tissues.
- Tissue microarray (TM) blocks were prepared from FFPES using 0.6 mm cores selected from the most representative tumor areas. Immunohistochemical analysis of 4 um TM sections was done by the standard IHC techniques with appropriate antigen retrieval for each antigen: ERα, PgR, AR, CyclinD-1, Her2/neu, p53, Ki-67, EGFR, CK5/6, NFk-B/p65 (abcam), TIF-2 (BD Biosci), AIB-1 (BD Biosci), p-c-Jun (Santa Cruz), SRC-1 (Cell Signal), p53 and ERβ (Biogenex, Thermo, Dako). ERβ mRNA expression was correlated with ERα mRNA and other biomarker variables, age, tumor size, stage, and grades.

1) ERβ1 primer set
   Sense : GATGCTTTGGTTTGGGTGAT
   Antisense : GGTCATACACTGGGACCACA
   Reference sequence : NM_001437 (1771-1936 : 166 bp)
2) ERβ2 primer set
   Sense : TGGCTAACCTCCTGATGCTC
   Antisense : ATGGATTACAATGATCCCAGAGG
   Reference sequence: NM_001040276(2107-2314: 208 bp)
   NM_001040275(1832-2039 : 208 bp)
3) ERβ5 primer set
   Sense : GTTTGGGTGATTGCCAAGAG
   Antisense : TTGCAGACACTTTTCCCAAA
   Reference sequence : DQ838583.1(1312-1496 : 185 bp)
4) ERα primer set
   Sense : TCCTCATCCTCTCCCACATC
   Antisense : TCTCCAGCAGCAGGTCATAG
   Reference sequence : NM_000125(1757-1861 : 105 bp)
5) ERβ total primer set
   Sense : CCTCAAAAGAGTCCCTGGTG
   Antisense : CTTCACACGACCAGACTCCA
   Reference sequence : NM_001437(772-970 : 199 bp)
6) beta-Actin primer set
   Sense : GATGAGATTGGCATGGCTTT
   Antisense : CACCTTCACCGTTCCAGTTT
   Reference sequence : NM_001101(1276-1375 : 100 bp)

Total RNA was extracted from FFPE human tissue using RecoverAll total nucleic acid isolation kit (Ambion). RT-PCR was performed using FastStart universal SYBR Green master Mix (Roche) and monitored using Eppendorf realplex 2. The PCR was carried out as following: 45 cycles at 95° C for 15 sec, 60° C for 45 sec. Reaction was done in triplicate. The data were analyzed using the comparative Ct method. The beta actin was used as a reference gene.

RESULTS

- ERβ isoforms were expressed in different types of BC with distinct patterns**.** ERβ2 was the major variant in both cancer tissues and breast cancer cell lines.
- A significant amount of ERβ expression was seen in ERα negative and TN, HER2 type, Luminal type B and basal-like BC.
- ERβ was co-expressed with ERα in > 50% of BC and with LAO another ERβ isoform(s). When co-expressed, ERα was up-regulated, and ERβ down regulated in the majority of BC and the ratio of ERα: ERβ ranged 3-100:1 (**Figure1**).
- Ki-67+ proliferating cells (>20% nuclear staining) were mostly in ERα- rather than ERα+ cases (69.0% vs. 31.0%) and were ERβ+, as were cyclin D1- cells (82.2% vs. 17.8%).
- In ERα negative tumors, ERβ expression was associated with increased expression of AIB-1 (41.1%), SRC-1 (37.3%), TIF-2 (52.8%), p-c-Jun (39.3%), NFk-B/p65 (48.3%) and p53 (39.3%). AR was expressed in 21.2% of TN and 42.6% of ERα- BC and was highly co-expressed with ERβ.
- During F/U period of 9 years, 18 patients were dead with diseases within 5 years from the date of diagnoses, and the predominant expression of ERβ isoform in these patients was ERβ2 (63.2%). ERβ isoforms were absent in 11.9% of all BC but absent in 30.0% of patients who died. Overall, ERβ mRNA expression did not show any significant correlation with age, tumor size, lymph node status and histological grades.

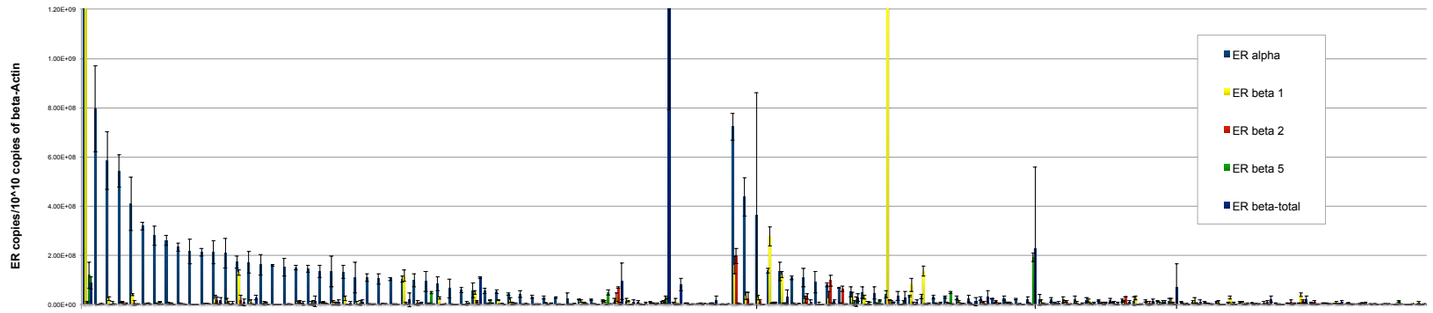

Figure 1. Relative Expression of ERα and ERβ Isoforms in Human Breast Cancer Tissues

ERβ Expression in Different Types of Breast Cancers

Types of Breast Cancers (# cases)	ERβ1 (%)	ERβ2 (%)	ERβ5 (%)	ERβ total (%)
ERα+ (72)	36.1	40.3	41.7	50.0
ERα- (69)	34.8	49.3	29.0	55.0
Triple Negative (ERα-/PR-/Her2, 43)	39.5	51.2	30.2	60.5
ERα+ and/or/PR+/Her2- (Luminal A type, 50)	36.0	40.0	38.0	50.0
ER- and/or PR+/Her2+ (Luminal B type, 21)	38.1	57.5	52.4	57.1
ERα-/PR-/Her2-/CK5/6+ (Basal-like type, 12)	50.0	58.3	33.3	58.3
ERα-/ PR-/Her2+ (HER2 Rich type, 18)	55.6	66.7	31.3	66.7
ER-/PR-/Her2-/AR+ (TN/AR+, 7)	57.1	85.7	14.9	100
Grade 3 (78)	41	46	31	34.6
Grade 2 (52)	39	54	29	51.9
Tumor size > 2cm (41)	31.7	53.9	33.9	46.3
Tumor size < 2cm (102)	43.9	34.7	38.8	39.8

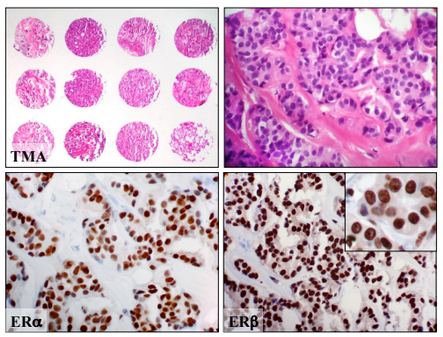

Figure 2. ERα and ERβ Expression in breast cancer

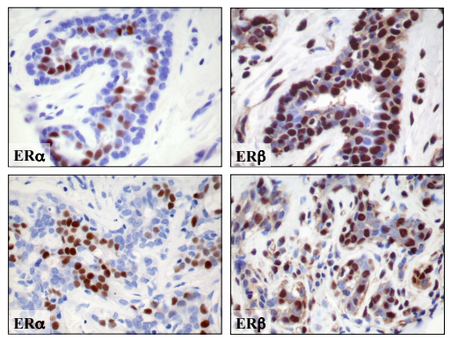

Figure 3. ERα and Erβ expression in benign breast tissue

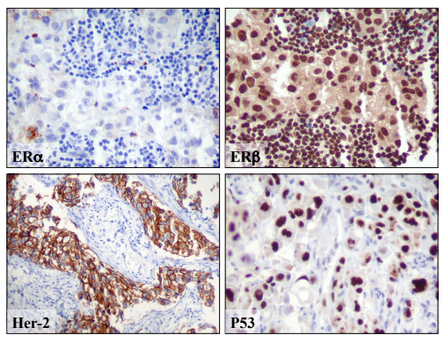

Figure 4. Correlation of ERα, ERβ, Her-2/neu and P53

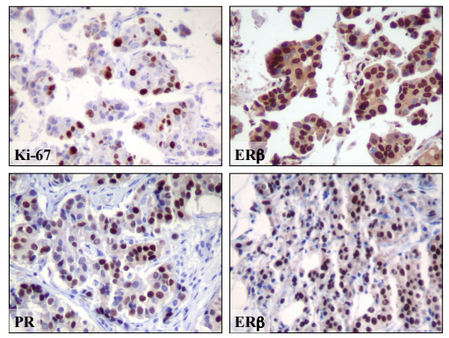

Figure 5. Correlation of ERβ, PR and Ki-67

CONCLUSIONS

- Each ERβ isoform was significantly and differentially expressed in ERα negative, TN, and basal-like and HER2 types, and associated with increased expression of nuclear co-activators/co-regulators and Ki-67.
- Highly proliferative ERβ+ BC cells may render them more sensitive to TAM and its metabolites as shown in previous studies. ERα-independent alternative mechanism of action of TAM may be via ERβ.
- When ERβ and ERα are co-expressed, ERβ appears to play a more distinct role than that in ERα negative BC, and the relative expression levels of ERα and ERβ could be the key determinants of cell responses to agonists and antagonists**.**
- Erβ has the potential to become a new therapeutic target and a treatment option in this specific sub-cohort of ERα negative BC patients.

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