
# Estrogen receptor beta modulates breast cancer cells functional properties, signaling and expression of matrix molecules

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## Abstract

Estrogen receptors have pivotal roles in breast cancer growth and progression. ERα has been clearly shown to play key role in hormone-dependent breast cancer properties, but little is known for the isoform ERβ. To evaluate the role of ERβ, we established stably transfected ERβ-suppressed MDA-MB-231 breast cancer cells by knocking down the human ERβ gene, using specific shRNA lentiviral particles. As observed by scanning electron microscopy, the ERβ suppression induces significant phenotypic changes in these cells, as compared to the control cells. Notably, the down-regulation of ERβ decreases the expression of the mesenchymal markers fibronectin and vimentin, whereas it increases the expression levels of the epithelial marker E-cadherin and cell junctions. These alterations are followed by reduced levels of the functional cell properties that promote the aggressiveness of these cells, such as proliferation, migration, spreading capacity, invasion and adhesion on collagen I. Notably, the down-regulation of ERβ reduces the migration of breast cancer cells through the tyrosine kinase receptors EGFR/IGF-IR and the JAK/STAT signaling pathways. Moreover, ERβ has a crucial role on the gene expression of several matrix mediators, including the proteoglycans syndecans-2/-4 and serglycin, several matrix metalloproteinases, plasminogen activation system components and receptor tyrosine kinases. These data clearly show that ERβ plays a crucial role in the cell behavior and ECM composition of the highly aggressive MDA-MB-231 cells and opens a new area of research to further understand its role and to improve pharmaceutical targeting of the non-hormone dependent breast cancer.



## 1. Introduction

Human breast cancer is a heterogeneous, complex malignancy accounting for the second leading cause of cancer death among women [1,2]. Estrogens have pivotal roles in the growth, development and progression of breast cancer. There are two genetically distinct and functional estrogen receptors (ERs), ERα and ERβ, belonging to the superfamily of nuclear receptors for steroid/thyroid hormones. The structural differences between the two ERs indicate that they serve distinct actions [3]. ERs can be regulated by extracellular signals, such as growth factors, acting independently from estrogens.

While ERα contribution in breast cancer growth and development has been thoroughly studied, the role of its isoform ERβ is less elucidated. ERβ is the second, genetically distinct ER subtype, discovered in 1995 and it has several alternatively spliced variants. Upon activation, ERβ can form homodimers and/or heterodimers with ERα [4], thus modulating the biological activity of ERα. It is documented that both ERα and ERβ present antagonistic actions, regulating the cell behavior in breast cancer initiation and

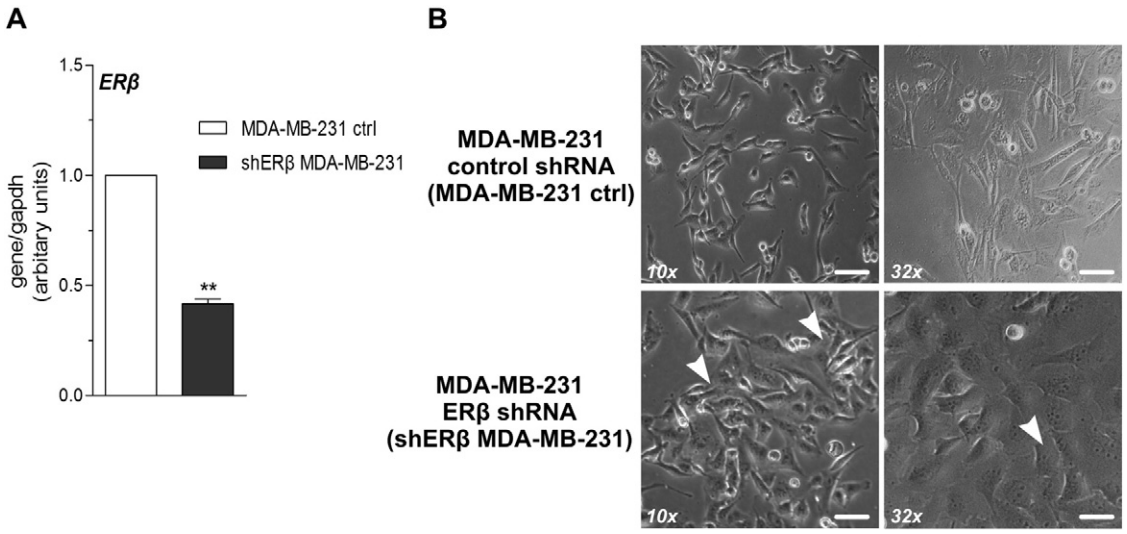


**Fig. 1.** ERβ suppression in MDA-MB-231 breast cancer cells. A, quantitative RT-PCR analysis of ERβ mRNA levels and B, phase-contrast microscopy to monitor cellular morphology of MDA-MB-231 cells infected with control lentiviral particles (MDA-MB-231 ctrl) and MDA-MB-231 cells transfected with ERβ shRNA lentiviral particles (shERβMDA-MB-231). Cell aggregates are shown with arrows (magnification 10× and 32×, bar 10 μm). Asterisk (**) indicates statistically significant differences ($p < 0.01$).

differentiation. Literature data suggest that ERβ is capable of reducing cell proliferation and tumor formation induced by ERα, while it can regulate reporter genes as well as endogenous gene expression [5,6]. Moreover, ERβ knock-out mice showed significantly reduced fertility in female, with ovaries exhibiting follicular arrest and anovulation [7]. Cancer progression involves different stages, including tumor growth, invasion, metastasis and angiogenesis. During breast cancer progression, cells become generally more aggressive and are characterized by cytoskeleton rearrangement, changes in cell shape and organization, loss of cell polarity and cell–cell adhesion and by the appearance of mesenchymal characteristics [8]. These changes lead to increased invasion ability, migratory capacity and resistance to apoptosis and as a result cells undergo epithelial-to-mesenchymal-transition (EMT) [9]. Under certain conditions, it is possible for differentiated cancer cells to regain polarity and establish more cell–cell adhesion contacts. This transition is characterized by the down-regulation of mesenchymal markers and transcriptional factors, such as fibronectin, vimentin and Snail, followed by the up-regulation of epithelial markers, such as E-cadherin [10,11].

It is well established that interactions among cancer cells and tumor microenvironment are in a dynamic interplay and are regulated by extracellular matrices (ECMs). ECMs are functional scaffolds consisting of specific macromolecules that facilitate in tumor growth and the initiation of invasion and metastasis [12,13]. Moreover, ECM components contribute to the maintenance of structural network and modulate cell signaling [14]. Among these molecules are the ECM remodeling enzymes, such as matrix metalloproteinases (MMPs), that are important players for microenvironment recycling, as well as proteoglycans (PGs), regulating tumor progression and cell signaling [8,13,15–17]. Thus, the importance of the interaction between the great variety of ECM biomolecules has been associated with the pathophysiology, cell properties and phenotype characteristics.

In earlier studies, we demonstrated the molecular basis under re-organization of ECM that seems to be influenced by the action of estrogens and their receptors in breast cancer [16,18]. Moreover, we have recently demonstrated that the induced loss of ERα in MCF-7 epithelial breast cancer cells results in EMT, striking changes in functional breast cancer cells' properties as well as in the expression patterns of certain ECM effectors [19]. Therefore, the aim of this study was to evaluate the role of ERβ, which is an integral component of the highly aggressive breast cancer cells MDA-MB-231, on functional cell properties and expression of ECM molecules implicated in cancer progression. Here, we demonstrate that the suppression of endogenous ERβ modulates changes in cell morphology and functional properties of aggressive breast cancer cells as well as changes in typical EMT markers and expression profiles of matrix biomolecules, emphasizing a novel role of this estrogen receptor in breast cancer cell behavior and properties.