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Elucidating the role of estrogen receptor-beta in triple negative breast cancer

Abstract

There are conflicting findings suggesting that estrogen receptor-beta (ERβ) has both proliferative and inhibitory effects on the pathogenesis of triple negative breast cancer (TNBC). A literature review of studies examining the viability of ERβ as a plausible molecular target for developing new TNBC therapies revealed that contrary findings regarding its functionality may be due to the heterogenous nature of TNBC and the type of cell lines used in various studies, discrepancies in the methodology and specificity of antibody testing, the varied expression of ERβ isoforms at the RNA and protein level, and how ERβ behaves in different races.

Introduction

Breast cancer is the second most common cancer among women in the United States and is clinically defined by the presence of three biomarkers: estrogen receptor-alpha (ERα), the progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). When present, these biomarkers can be assessed to predict prognosis as well as the potential response to endocrine treatments that add, block, or remove hormones (1, 2). Triple negative breast cancer (TNBC) is a subtype of breast cancer that is defined by the absence of these three biomarkers (3). However, while ERα is not expressed in TNBC, ERβ is expressed in about 60%–80% of TNBCs (4). And, although TNBC accounts for 15-20% of all breast cancer cases, it is the cause of over 50% of breast cancer mortality due to its aggressive nature and limited treatment options as the

lack of hormonal receptors makes the development of targeted therapies more challenging (1, 3). TNBC also appears to demonstrate a resistance to radiation, and the TNBC subtype has been associated with higher risk of local regional recurrence and contralateral disease, as well as systemic relapse (5). For these reasons, identifying novel therapeutic strategies that can provide more effective and successful treatment for patients with TNBC is of critical importance (1).

Estrogen receptors are ligand-activated transcription factors that play different roles in gene regulation and show both overlapping and specific tissue distribution patterns. ERα is the major driver of approximately 75% of breast cancers and its role in breast cancer has been extensively studied. As such, ERα and ERα-regulated genes represent the main targets in clinical approaches aimed to control hormonally responsive breast cancer. However, the exact role of ERβ in carcinogenesis and tumor progression is not fully understood. ERβ has several isoforms (ERβ1, ERβ2/cx, ERβ3, ERβ4, and ERβ5) and has been reported to display both highly variable and contrary effects, including both proliferative and inhibitory capabilities. Several studies have found that decreased ERβ expression correlated with tumor progression and invasiveness (6, 7). On the contrary, Austin et al. (2018), found that ERβ activation is pro-tumorigenic in nature (3).

I hypothesize that ERβ is a plausible molecular target for the development of therapies for the treatment of TNBC. The goal of this literature review is to determine if current evidence outlining the anticancer effects of ERβ is sufficient to pursue ERβ as a molecular target for the development of new and effective TNBC therapies, despite conflicting findings. The objectives of this paper are to 1) elucidate the role of ERβ in TNBC proliferation, invasion and migration, 2) explain possible reasons for conflicting findings regarding the role of ERβ in TNBC progression.

Methods

Methods used to locate relevant literature on the role of ERβ in TNBC pathogenesis included searching for peer-reviewed scientific research publications via Google and Google Scholar. Open access databases provided a list of additional relevant literature on the subject.

Results

An increasing body of evidence suggests that ERβ is a protective factor that suppresses uncontrolled proliferation, which is mediated by concentration-dependent and cell line-specific effects on cell growth and gene expression. In addition, ERβ can exert its antitumor effect via gene transcription and miRNA regulation (8). In a 2014 study by Hinsche et al., investigators sought to demonstrate whether ERβ agonists reduced the invasiveness of TNBC cells. Study results showed that cell invasion of HCC1806 and HCC1937 TNBC cells was significantly increased when co-cultured with MG63 osteoblast-like cells. However, treatment with ERβ selective estrogen agonists liquiritigenin and ERB-041 reduced the ability to invade a reconstituted basement membrane and to migrate in response to the cellular stimulus. Yet, both ERβ agonists were found to have no effects on TNBC cell proliferation. In control experiments treatment with 17β-estradiol resulted in a slight increase of proliferation of TNBC cells (7). However, ligand-mediated anti-proliferative effects of ERβ1 have been demonstrated in TNBC cell lines MDA-MB-231 and Hs578T, where ectopic ERβ1 inhibits cell proliferation rate after treatments with 17β-estradiol or ERβ specific agonists such as FERb 033, and liquiritigenin (2).

Hinsche et al. (2014) also examined the effects of ERβ agonist treatment on CXCR4 protein expression. The CXCR4 protein helps to regulate cell growth, proliferation and migration. When TNBC cells were co-cultured with MG63 cells, CXCR4 protein expression of TNBC cell lines HCC1806 and HCC1937 was significantly increased. However, treatment with ER agonist liquiritigenin resulted in a significant decrease of CXCR4 protein expression (7).

In a 2016 in vitro study conducted by Schüler-Toprak et al., invasiveness of MBA-MB-231 and HS578 breast cancer cells decreased after treatment with ERβ agonists ERB-041 and WAY200070. Agonists liquiritigenin and 3β-Adiol only reduced invasion of MDA-MB-231 cells. Knockdown of ERβ expression increased invasiveness of MDA-MB-231 cells about 3-fold (9).

A 2019 study by Song et al. found that ERβ inhibits breast cancer cells migration and invasion through Claudin-6 (CLDN6)-mediated autophagy. CLDN6 is a tight junction protein that acts as a tumor suppressor gene in breast cancer. Study results demonstrated that 17β-estradiol upregulated the expression of CLDN6, which was mediated by ERβ which regulated CLDN6 expression at the transcriptional level and inhibited the migration and invasion of breast cancer cells through CLDN6 (6).

Using a cohort of TNBC patients extensively characterized at the DNA and RNA level, Reese et al. (2018) demonstrated that therapeutic activation of ERβ elicits powerful anticancer effects in TNBC through the induction of a family of secreted proteins known as the cystatins, which function to inhibit transforming growth factor (TGFβ), signaling and suppressing metastatic phenotypes both in vitro and in vivo. Ligand-mediated activation of ERβ with estrogen or LY500307 resulted in decreased invasion and migration of TNBC cells in vitro and prevented the formation of lung metastasis in vivo (1).

Despite significant evidence that ERβ may be a promising molecular target for development of TNBC therapeutic interventions, there have been conflicting findings suggesting that ERβ has cancer promoting capabilities. Austin et al. (2018) found that ERβ activation is pro-tumorigenic in vitro. TNBC cell lines reviewed for that study showed that activation of ERβ results in increased secretion of insulin-like growth factor (IGF2) which can bind to IR/IGF1R,

and activate growth promoting capabilities. The authors also demonstrated that in most TNBC cells, activation of ERβ by diarylpropionitrile (DPN) significantly increased cell invasion and migration, especially in cells that are not highly invasive (MB468, HCC-70/1806). However, in MB-231 cells, DPN caused significant decrease in cellular invasion (3).

Mukhopadhyay and colleagues explored whether tumor protein p53 (TP53) status could be a determinant of whether ERβ expresses anti-proliferative or proliferative properties in TNBC. This protein acts as a tumor suppressor by prohibiting cells with mutated or damaged DNA from dividing. The study findings showed that ERβ interaction with wild-type and mutant TP53 cause pro-proliferative and anti-proliferative effects (4). Table 1 shows the role of ERβ in TNBC clinical outcomes (8).

Table 1. The role of ERβ in clinical outcomes

ERβ isoform	ERβ expression	ERα status	Number of patients	Clinical outcome
ERβ	↑	–	1400	reduced RFS
ERβ			32	worse prognosis
ERβ	↑	+/–	1026	better prognosis
ERβ	↑		120	worse prognosis
ERβ		–	17	no association with PFS
ERβ	↑	+	195	reduced DFS; reduced DFS after endocrine therapy
Nuclear ERβ1		–	126	no association with DFS and OS
ERβ	↑	+	127	no association with PFS
ERβ	↑	–	107	reduced DFS
Nuclear ERβ1	↑	–	19	reduced OS
ERβ1		–	571	prolonged OS, DFS, and DMFS
ERβ	↑	+/–	583	worse prognosis; worse endocrine therapy response
ERβ	ERα/ERβ: 1–1.5		78	better hormonal treatment response
ERβ	↓		89	reduced OS
Nuclear ERβ1	↑	+/–	123	better chemotherapy therapy and endocrine therapy response
Cytoplasmic ERβ 2/cx		+/–	123	poor chemotherapy response
ERβ	↓		41	prolonged PFS; better aromatase inhibitor therapy response
Nuclear P-S105-ERβ	↑	+/–	459	better prognosis

RFS Recurrence-free survival, *PFS* Progression-free survival, *DFS* Disease-free survival, *OS* Overall survival, *DMFS* Distant metastases-free survival.

Discussion

ERβ signaling is a complex and multifaceted phenomenon and not just a component of a linear signaling pathway (8). Yet, there is conflicting evidence regarding the exact role that ERβ exerts and whether it inhibits cancer progression or facilitates the pathological process. There are

several factors that may explain the conflicting findings regarding the role of ERβ in cancer development. First, the type of TNBC cell lines used in studies could be a factor in how ERβ behaves. According to Austin et al., although TNBC is a heterogenous disease, most studies that examine TNBC used more accessible TNBC cell lines (MB231 and Hs578T) and did not account for the heterogeneity of TNBC which has four subclasses (basal-like, mesenchymal, immune enriched, and luminal AR). Hence, while Austin and colleagues found that ERβ activation caused decreased invasion in the MB-231 cell line, its activation did not inhibit cell migration. Also, in mesenchymal subtype BT-549, ERβ activation increased cellular invasion. These findings support the idea that ERβ could have different effects in specific subtypes of TNBC (3).

Second, ERβ has multiple isoforms and they have been shown to express themselves differently in breast cancer at both the RNA and the protein level. For instance, in TNBC patients, high levels of the isoform ERβ2/cx have been associated with early tumor recurrence. Furthermore, similar behavior of this isoform has also been observed in TNBC cell lines, where ERβ2/cx, altogether with ERβ4-5 isoforms, enhances hypoxic signaling, previously correlated to tumor aggressiveness (2). Also, the ERβ4 isoform, that is not expressed in physiological conditions, has been correlated with poor outcome in TNBC patients. These findings suggest that ERβ isoforms have specific and varied involvement in tumor development and may partially explain some of the contradictory evidence regarding the ERβ role in cancer.

The third factor that may explain conflicting findings regarding the role of ERβ is the processes surrounding the adequate validation of the specificity of commercially available antibodies, and the lack of standardization of immunohistochemistry protocols and tissue samples preparation (2). Nelson et al. reported marked variation in the ability of commonly used commercially available ERβ antibodies to accurately detect ERβ by Western blotting and protein

purification-MS based methods (10). There has also been discordant evidence reported for other commonly used antibodies. In a study that explored the monoclonal PPG5/10 which only recognizes ERβ1 isoform, its reliability in immunohistochemistry analysis in breast cancer cells and tissues was affirmed, yet other studies found it incapable of specifically detecting the receptor via Western blot. These discrepancies may not only be caused by the specificity of the antibodies but also by differences in experimental techniques employed (2).

Finally, race may be a factor in how ERβ is expressed in TNBC. Black women are more likely to present with more advanced stages of TNBC than White women, have higher incidence rates, and are more likely to have TNBC that has metastasized to other parts of the body. Austin et al. found that ERβ and IGF2 are expressed significantly higher in Black and Hispanic women when compared to White women and that IGF2 and ERβ expressions correlated with each other. It is believed that this co-expression may result in decreased overall survival of TNBC patients (3). Therefore, further examination of the role of race in the behavior of ERβ is warranted.

It appears that the mechanisms underlying the role and actions of ERβ in TNBC progression, proliferation, invasion and migration are complex, diverse, and may be specifically impacted by various genetic and molecular factors. ERβ seems to be a viable target for new TNBC therapy development but further examination into how this receptor functions in different breast cancer subtypes, investigation of the behavior of various ERβ isoforms, rigorous testing to validate ERβ antibodies, and exploration of how ERβ behaves in different races is warranted.

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