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Molecular Roles of *GREB1* in ER-Positive Breast Cancer

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Abstract

Growth regulation by estrogen in breast cancer 1 (*GREB1*) is one of the top estrogen (E2) responsive estrogen receptor (ER) target gene. *GREB1* play pivotal role in the ER signaling dependent oncogenesis of breast cancers. *GREB1* has been reported as a regulatory factor in ER signaling as it interact and regulate the function of ERα; the predominant subclass of ER. *GREB1* acts as transcription coactivator that affects ER-chromatin interaction thereby modulate its downstream oncogenic signals that initiate the development and progression of breast cancer. Such intimate role of *GREB1* places it as a therapeutic target and clinical biomarker for patient's response to endocrine therapy. More recently Tamoxifen resistance in breast cancer was found to be regulated by the EZH2-ERα-*GREB1* transcriptional axis. Despite the presence of such discrete evidence on the involvement of *GREB1* in triggering the oncogenesis as well as drug response of breast cancer, there is very few compiled reports on the possible molecular mechanism how *GREB1* could affect ER-associated tumor growth and subsequent therapeutic responses. Hence this review is written on the molecular roles of *GREB1* in ER-positive breast cancer.

Keywords: *GREB1*; Breast; Cancer; Estrogen receptor alpha; Oncogenesis

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Introduction

ER-positive breast cancer is the most common type of breast cancer. It constitutes more than 75% of breast cancers. *GREB1* is highly expressed in ER-positive breast cancers and it is one of the E2-induced ER target genes [1-4]. Knockdown of *GREB1* results in inhibition of cell proliferation in several hormone-dependent cancers. Recently, *GREB1* was reported to function as a transcription co-activator of ERα. Loss or dysregulation of *GREB1* substantially decreased ERα-mediated gene transcription and reduced tumor growth [1,2,4,5]. One study found *GREB1*-ERα interactions in 50% of ER+ cancers and showed *GREB1* expression was correlated with a good clinical outcome [1]. Nevertheless, little is known about the exact role of *GREB1* in the cascade of hormone action, though it appears to be a key E2-induced gene having role in ER signaling.

In ER signaling pathway, E2 induces the expression of ER target genes with a subsequent effect on cancer cell growth, survival and metastasis. *GREB1* is well known ER target gene reported to be critically involved in the E2-induced breast cancer growth and as a clinical marker for patients' response to endocrine therapy [1]. More recently *GREB1* was reported to be involved in modulating ER signaling in which si*GREB1* affect ER-chromatin interaction [5]. Despite the fact that *GREB1* is well known as top E2 responsive ER target regulating ER signaling, there is very few reports on the detailed molecular mechanism how *GREB1* affect ER-associated tumor growth and subsequent therapeutic responses. This review is thus elucidates the molecular roles of *GREB1* in the oncogenesis as well as drug response of ER-positive breast cancer.

Literature Review

Genomic location of *GREB1* and its Isoforms

GREB1 is encoded on the plus strand of chromosome 2, at 2q25.1, covering 108.67 kb from 11674242 to 11782912 **(Table 1).** The gene structure of *GREB1* consists of 60 alternative exons and 40 different introns. The partial *GREB1* sequence was first cloned from the size-fractioned adult brain cDNA library in 1998 and named KIAA0575 [6]. Two years later, the three primary representatives *GREB1* complete cDNA clones were isolated from an ER+ breast cancer cell line (MCF7) cDNA library and named as *GREB1a, GREB1b* and *GREB1c* respectively [7]. The three transcript variants contain distinct c-terminus regions on genomic locus; differ in the number of exon and a total base pair **(Table 1).** More splice variants have been also reported in breast, uterus, prostate and brain [6,8].

Table 1 Sequence organization of the three isoforms of *GREB1.*

Isoforms	Genomic location	Accession	Length (aa)	Molecular mass (KDa)	Exon	Base pair
GREB1a	11674242-11782912	NP_055484	1949	216.5	33	8482
GREB1b	11682851-11732446	NP_149081	457	48.9	11	2521
GREB1c	11679969-11728353	NP_683701	409	43.2	10	2432

The *GREB1* gene contains three consensuses EREs motif located at 1.5 kb, 9.5 kb, and 21 kb upstream of the transcription start site (TSS) [9]. The ERE found at 1.5 KB upstream of TSS is considered as a *GREB1* core promoter where ERα binds. The ERE found 9.5 KB upstream of TSS and the 21 kb distal enhancer cooperate for transcriptional induction of the *GREB1* gene by ERα through making chromatin loops. In breast cancer cells, the steroid receptor co-activator SRC-3, phosphorylated RNA polymerase II and acetylated histones are also bound to these ERα-ERE complexes in the presence of E2 as evidenced by chromatin immunoprecipitation (ChIP) assay [10,11]. These findings on the genomic location of *GREB1* indicate that *GREB1* is an ERα target gene.

Oncogenic roles *GREB1* in ER-positive breast cancer

The oncogenesis of breast cancer has been associated with ERα; the predominant subclass of ER in ER signaling pathway. ERα play its oncogenic role by causing deranged activation its target genes. Among the ER target genes, *GREB1* have been reported to be involved in the E2-induced breast cancer growth, survival and metastasis. This is evidenced by the presence of positive correlation ERα-positivity of breast cancer cell lines and *GREB1* mRNA and protein expression as well as chromatin binding study so far conducted shows *GREB1* as a downstream target of ERα [1,5]. This pattern also correlates with the progression of various hormone-dependent cancers such as breast, ovary, and prostate cancers [1-4]. On top of this it has been shown that *GREB1* knockdown significantly reduces tumor growth [1,2,4].

Mechanistically *GREB1* was found to function as a transcription co-activator of ERα by bridging ERα and other transcription activators such as CREB-binding protein/P300 histone acetyltransferases thus it mediate interactions between the ER and additional proteins through which it affects the expression of E2-induced oncogenic ER target genes. In the same study, the removal of *GREB1* substantially decreases ERα-mediated gene transcription and cell proliferation [5]. Upon *GREB1* knockdown in MCF-7 cells, nearly half of the estrogen- responsive genes were no longer differentially expressed, and the cells were less able to form colonies [5]. In further elucidation of the above mechanism compelling reports have shown the presence of *GREB1* sensitizes E2-responsive breast cancer to endocrine therapy [12]. Consequently, it indicates that on top of the conventional ER-targeted therapy, *GREB1* could be a potential therapeutic target for hormone sensitive ER-positive breast cancer.

In the oncogenesis of breast cancer, E2 influences uncontrolled cell proliferation or promote ER independent signaling to sidestep the physiologically controlled ER signaling [13,14]. Few reports also showed that breast cancers express a protein that stabilizes ERα to promote proliferation [15,16]. Since *GREB1* is a well characterized E2-responsive gene used to identify ERα activity and silencing *GREB1* can affects the ER oncogenic signatures known to regulate the proliferation of breast cancer cells and its phenotypic properties, further detail molecular mechanism how *GREB1* play this role or weather *GREB1* reported to bind ERα could regulate ERα stability thereby ERα function is remain to be elucidated [5,17-20].

GREB1 modulate tamoxifen response of ER+ breast cancer

More recently loss of *GREB1* has been reported to be involved in the resistance to ER-targeted endocrine therapy. As *GREB1* is an ER target gene, persistent ERα targeted tamoxifen treatment could leads to gradual loss of *GREB1* in with those initially hormone sensitive ER-positive cells rather start to rely on the alternative mechanism for growth and survival **(Figure 1).** Beside this, *GREB1* has been depicted to mediate interactions between the ERα and other proteins, as an inhibitory factor in tamoxifen-liganded ER complex [5].

On top of this, patient's correlation study showed that the expression of *GREB1* is epigenetically silenced by enhancer of zeste homolog 2 (EZH2) in Tamoxifen-resistant cases [21]. Yet these findings indicate that maintenance of *GREB1* protein level is important to balance the ERα- dependent transcriptional responses. As indicated in **Figure 1** right panel, EZH2-medited epigenetic silencing decreases the expression *GREB1* thereby trigger Tamoxifen resistance. Indeed, the role of epigenetic regulation in E2-responsiveness has come to light as a potential treatment target to revert hormone therapy-resistant breast cancers [22,23]. However, the detailed mechanism how the presence of *GREB1* sensitizes the cells and the reason why *GREB1* needs to be downregulated for the acquisition of endocrine resistance remains unclear suggesting the need for further study for the mechanistic role of *GREB1* in the pathophysiology of breast cancer.

Nevertheless, ERα is a master regulator and a well-validated drug target for a majority of breast cancers, yet the mechanisms by which agonist-bound ERα causes repression and the mechanism of resistance to such drug action are poorly understood. Although *GREB1* is known to bind to ERα and associated effects were also consistent across those studies, the direct effect of *GREB1* binding on ERα is yet to be disclosed.

GREB1 as biomarker in the breast cancer

Tumor ERα status is routinely determined as it predicts for response to hormonal therapy. However, the predictive power of ERα alone is limited and tumor ERα status fails to identify those more likely to recur after hormonal therapy (suggestive of de novo

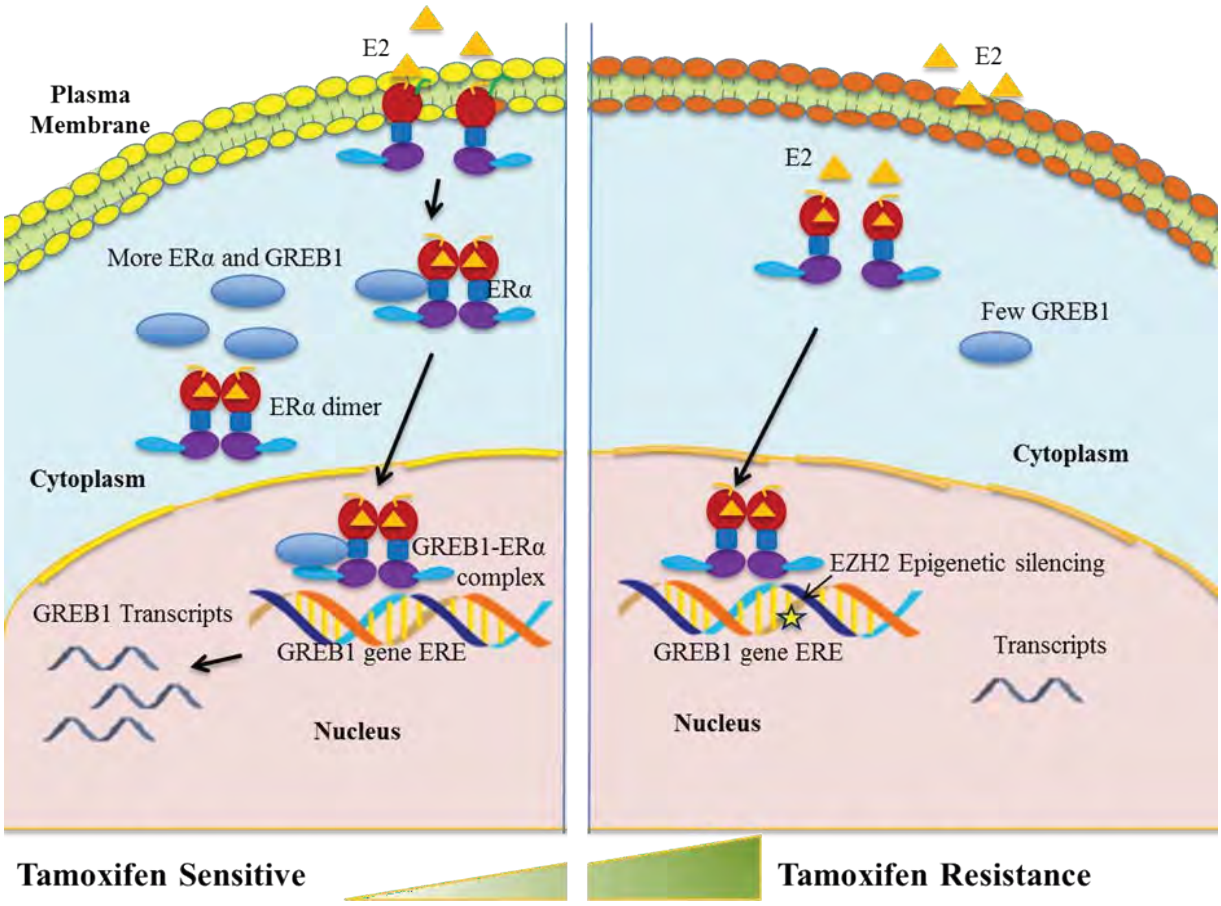

Figure 1 **Roles of *GREB1* in E2-induced ER signalling pathways of ER-positive cells**. *GREB1* gene has ERE where ERα binds and induces its expression. Tamoxifen sensitive cells have more GREB1 expression which subsequently interacts with ERα (Left Panel) to further boost its own expression. While in Tamoxifen resistant cells, EZH2-medited epigenetic silencing decreases the expression *GREB1* (right panel).

or acquired endocrine resistance) [24,25]. This may be related to the way in which ERα positivity is defined. IHC is routinely used for evaluation of ERα, and a positive result is defined according to scoring systems that take into account staining intensity and proportion of positively-stained cells. In the case of ERα, several scoring systems are used and since the positive threshold for each differs, the definition of ER-positivity is somewhat ambiguous [26-28]. Furthermore, this threshold can sometimes be modified [29]. Low PR expression was an independent predictor of relapse, as was high Ki67 expression [30,31]. As a result, biomarkers like PR, major ERα target genes or downstream effector arms of ERα activation, and Ki67 (a marker of proliferation) have been evaluated in combination with ERα [32].

Because of the lack of perfect correlation between ER and/or PR status and patient's response to hormone therapy, there was a need to identify additional protein markers that would improve prediction of hormone response. *GREB1* has been reported as potential molecular markers not only in breast cancer but also for prostate cancer prognosis and more exceptionally in saliva samples for lung cancer [5,7,33,34]. On top of this *GREB1* with Paired-box gene, 8 (PAX8) has been depicted as a biomarker in ovarian cancer [34]. Concurrently same groups also depicted *GREB1* to underlie E2 induced progression of ovarian tumor and thus may act as a targetable molecule [4]. These all findings illustrate that *GREB1* as an effector arm of ER signaling is critical to improve prognostication.

Conclusion

GREB1 is one of the top E2 responsive ER target gene. It play pivotal role in the ER signaling dependent oncogenesis of breast cancers. Mechanistically *GREB1* interact and regulate the function of ERα. As transcription coactivator, *GREB1* affects chromatin interaction of ERα thereby regulates the expression of oncogenic signatures known to trigger the development and proliferation of breast cancer cells and its phenotypic properties. In this regard *GREB1* has been depicted to be used as therapeutic target and clinical biomarker for patient's response to endocrine therapy. On top of this loss of *GREB1* has been reported to be involved in the tamoxifen resistance in breast cancer. Such evidence on the involvement of *GREB1* in triggering the tumor growth as well as its drug response, suggests the need for detail study on the molecular mechanism how *GREB1* could affect ERα function

and ER-associated oncogenesis and subsequent therapeutic responses of ER-positive breast cancer.

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Conflicts of Interest

The authors declare no conflicts of interest.

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