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Prognostic and Predictive Significance of Eukaryotic Elongation Factor 1D (eEF1D) in Breast Cancer: A Potential Marker of Response to Endocrine Therapy

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Abstract: Components of the protein synthesis machinery are subjected to alterations in cancer cells. eEF1D gene, which lies within the frequently amplified 8q24 locus, is one of the subunits of the human eukaryotic elongation factor complex. This study aimed to evaluate the prognostic and predictive significance of eEF1D in breast cancer using *in silico* analysis tools. For this purpose, we analyzed genomic alterations of the eEF1D gene using TCGA datasets via cBioPortal. Histopathological analysis was performed on patient tissue images obtained from cBioPortal and the Human Protein Atlas. Survival analysis was carried out using the KM Plotter and the prediction of response to therapy was assessed via the ROC Plotter. We found that eEF1D was highly amplified and overexpressed in breast invasive carcinoma. Increased expression of eEF1D was correlated with increased structural disorganization and morphological alterations at the cellular level, as well as a shorter period of overall survival. Furthermore, prediction of response to therapy showed that patients with eEF1D overexpression responded significantly better to endocrine therapy. In conclusion, our results suggest a cancer-promoting role for eEF1D, associated with poor prognosis and points towards its predictive value in providing better tailored treatment options for a patient.

Keywords: eEF1D; cancer; prognosis; predictive biomarker; histopathology; endocrine therapy

1 Introduction

The genetic information within a cell is transcribed into RNA and translated into proteins in order to maintain proper cellular function. In eukaryotes, translation is completed in three steps: initiation, elongation and termination. Translation initiates with the assembly of the initiating tRNA carrying methionine, 40S–60S ribosomal subunits and eukaryotic initiation factors (eIFs) at the start codon [1]. The nascent polypeptide chain elongates as the ribosome moves further down the mRNA and new amino acids are added. Transfer of aminoacyl-tRNAs to the elongating ribosome is mediated by the eukaryotic elongation factors (eEFs) [2]. Translation terminates when the ribosome comes across a stop codon and the newly synthesized protein gets released [3].

The human eEF1 complex consists of eEF1A, eEF1B, eEF1D and eEF1G subunits. eEF1A catalyzes the GTP-dependent transfer of the aminoacyl-tRNAs to the ribosomal A-site, while eEF1B, eEF1D and eEF1G serves to recycle the GDP-bound inactive eEF1A into the GTP-bound active eEF1A to facilitate another cycle of elongation [2]. In addition to their critical roles during the process of protein synthesis, non-canonical roles have also been attributed to the eEF1 complex members in protein degradation [4,5]

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apoptosis [6,7] and oxidative stress response [8]. Elongation factors of the host are often hijacked during viral infections to assist the synthesis of viral proteins and some eEF1 complex subunits are implicated in viral replication [2,9]. Recent studies linked eEF1 complex proteins with cancer and epigenetic regulation mechanisms as well [10,11].

There is only a limited number of studies investigating the non-canonical functions of eEF1D in the literature. eEF1D, also known as guanine nucleotide exchange protein, is located to the long arm of chromosome 8 (8q24), which is one of the most frequently amplified genomic loci in cancer [12]. In line with this, eEF1D overexpression has been reported in several types of cancer and implicated in promotion of cell proliferation [11,13–15]. Studies so far has focused mainly on investigating established oncogenes and proto-oncogenes that lie within the 8q24 locus such as MYC in breast cancer [16], while the potential impact of copy number variations in eEF1D gene has remained to be solved. Therefore, in this study we aimed to evaluate the prognostic significance of eEF1D in breast invasive carcinoma (BRCA) and its potential as a novel oncogene.

2 Methods

2.1 Identification of Genomic Alterations

Copy number variations and the mRNA expression levels of eEF1D were analyzed using the TCGA PanCancer Atlas dataset for breast invasive carcinoma and visualized by the cBio Cancer Genomics Portal (http://cbioportal.org) [17]. Complete samples with mutation, copy number alteration and expression data were selected for the analyses.

2.2 Histopathological Evaluation

Images of hematoxylin and eosin stained BRCA patient samples were retrieved from the cBioPortal. Patients were classified into three groups according to their eEF1D status (amplified/deleted/no alteration). Three patients per group were randomly selected and analyzed. eEF1D protein levels were evaluated using anti-eEF1D antibody stained immunohistochemistry images of breast ductal and lobular carcinoma tissue samples available on the Human Protein Atlas (https://www.proteinatlas.org) [18].

2.3 Survival Analysis

Kaplan-Meier survival analysis was performed on KM Plotter (https://kmplot.com/analysis/) [19] using the best JetSet probe set for eEF1D (Affymetrix ID: 203113_s_at). The effect of eEF1D mRNA expression levels on overall survival was assessed on 1764 breast cancer patients using default settings. *p*-values below 0.05 (%5) were considered significant.

2.4 Prediction of Response to Therapy

The correlation between eEF1D expression levels and the response to the main breast cancer therapies were determined using the ROC Plotter (http://www.rocplot.org/) [20], which is an online predictive tool based on transcriptomic data of more than 3,000 breast cancer patients. Pathological complete response *vs.* residual disease after completing the therapy was analyzed on 1775 patients in relation to eEF1D expression levels (Affymetrix ID: 203113_s_at). The predictive potential of eEF1D gene as a biomarker was automatically calculated by the ROC Plotter. Statistical analyses were based on Mann-Whitney test and $p < 0.05$ was considered significant.

3 Results

3.1 eEF1D is Highly Amplified and Overexpressed in BRCA

To evaluate the oncogenic potential of eEF1D in breast cancer, we first analyzed the genomic alterations of eEF1D gene in a single patient basis. Overall, 12% of the patients within the dataset had eEF1D alterations, most of which was amplification events resulting in increased mRNA expression (Fig. 1a). Major subtypes of breast cancer within the patient cohort was breast invasive ductal carcinoma (72.0%) and breast invasive lobular carcinoma (18.5%), while the number of patients diagnosed with other subtypes was limited (Fig. 1b). Although amplification was the main genomic alteration event in all major breast cancer subtypes, we detected only three patients with homozygous eEF1D deletions (0.3%); all of which were diagnosed with ductal carcinoma (Fig. 1c). For this reason, for the histopathological analyses, we randomly selected three ductal carcinoma patients from each group of distinct eEF1D status (amplified/deleted/unaltered) and evaluated the hematoxylin and eosin stained tissue images. We found that regardless of the eEF1D status, all analyzed tissues displayed an infiltrative pattern (Fig. 1d). All tissues had high levels of cell proliferation, though it was more pronounced in eEF1D amplified and unaltered groups in comparison to the eEF1D deleted group. Furthermore, we observed increased tubular formation and presence of intact ductal structures in tissues with eEF1D deletions.

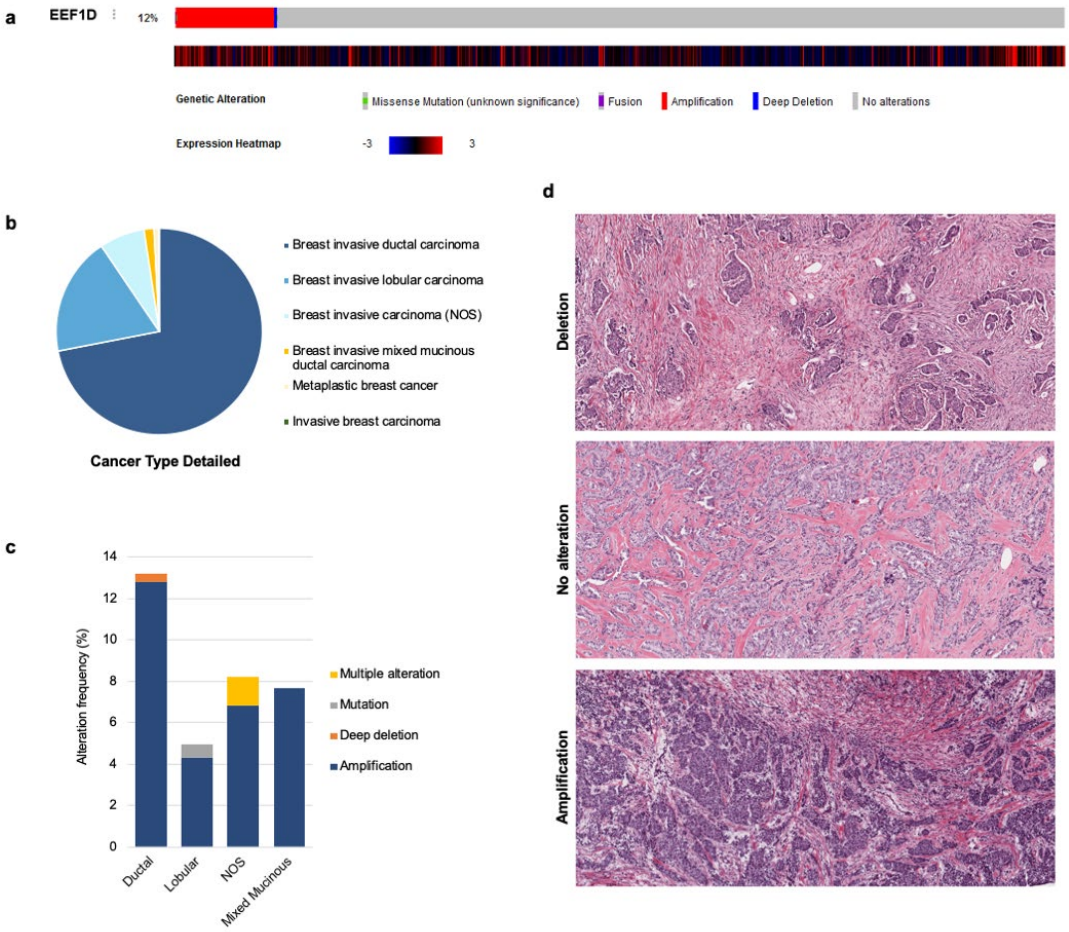

Figure 1: Genomic alterations in eEF1D gene (a), detailed cancer types (b) and the distributions of eEF1D alterations among the cancer subtypes (c) within the analyzed TCGA patient dataset. Representative images of hematoxylin and eosin stained BRCA patient samples with deletion (patient ID: TCGA-BH-A203), no alteration (patient ID: TCGA-E9-A1R7) and amplification (patient ID: TCGA-A8-A06T) in eEF1D are shown (d)

There were a total of 23 immunohistochemistry images from 12 patients available for analysis. Of these tissue samples, seven of them were highly stained for eEF1D, indicating high protein expression (Fig. 2). All patient samples with high expression of eEF1D were diagnosed with ductal carcinoma, while two tissues had medium staining in the same subtype. In patient samples diagnosed with lobular carcinoma, two had medium and one had low levels of antibody staining. In terms of histopathological analysis, we observed increased structural disorganization and morphological alterations at the cellular level in tissue images that had high antibody staining for eEF1D (Fig. 2, lower panel).

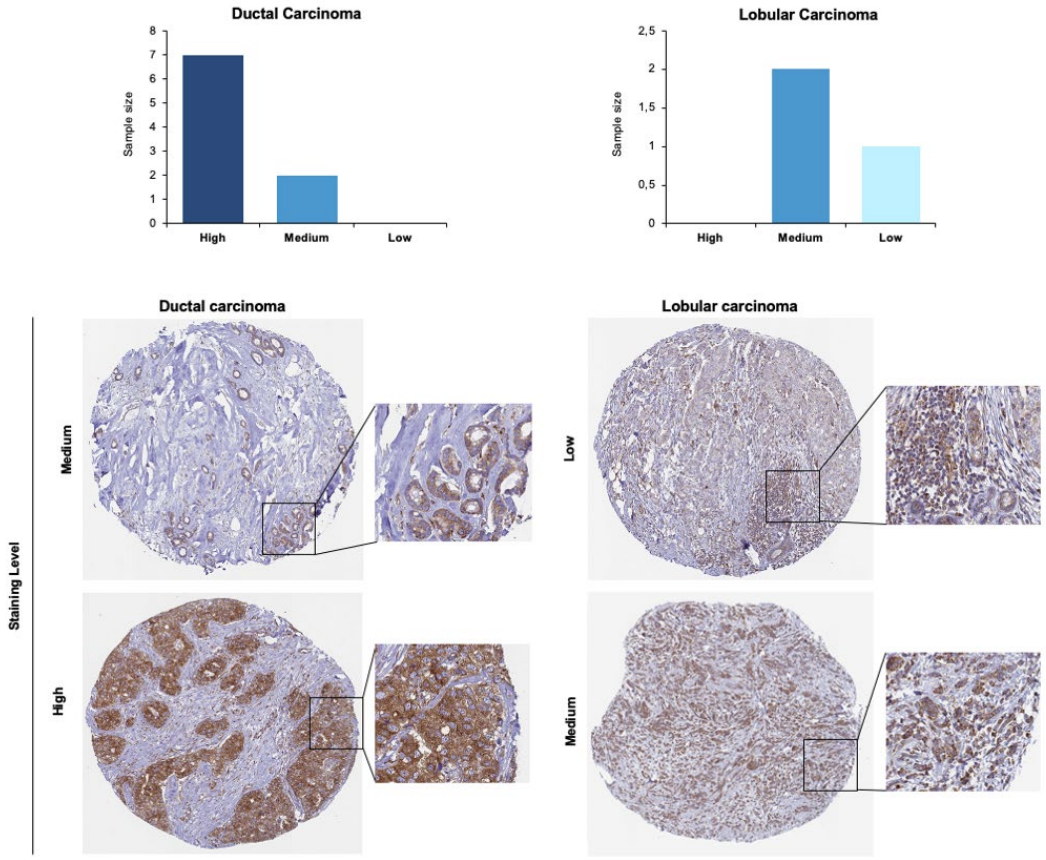

Figure 2: Quantification of eEF1D protein expression levels based on immunohistochemistry staining intensities in breast ductal and lobular carcinoma tissue samples (upper panels). Representative images of high, medium and low levels of eEF1D are shown in the lower panels. Patient IDs are given at the bottom-left corner of each image

3.2 Prognostic and Predictive Significance of eEF1D

After establishing that eEF1D gene is amplified and overexpressed in breast cancer, we set out to investigate its prognostic significance and predictive value. Analysis of relapse-free survival in relation to eEF1D mRNA expression levels in breast cancer indicated that patients with higher eEF1D expression had a shorter period of survival; suggesting poor prognosis (Fig. 3, $p < 0.01$).

Next, we analyzed the pathological complete response *vs*. residual disease after completing chemo-, endocrine or anti-HER2 therapy in relation to eEF1D expression levels. Fig. 4 shows that patients with eEF1D overexpression responded significantly better to endocrine therapy and to a lesser degree to chemotherapy (Fig. 4 upper panels, $p < 0.01$). On the other hand, eEF1D overexpressing patients responded poorly to anti-HER2 therapy, though the difference was not significant ($p = 0.18$). We also attempted to

determine the potential of eEF1D gene as a predictive biomarker in breast cancer using the ROC Plotter. The ROC Plotter returns an AUC (area under the curve) value between 0.5 (no predictive value) and 1 (perfect predictive biomarker) for a given queried gene, which identified the highest AUC value for eEF1D in breast cancer as 0.671 for responsiveness to endocrine therapy.

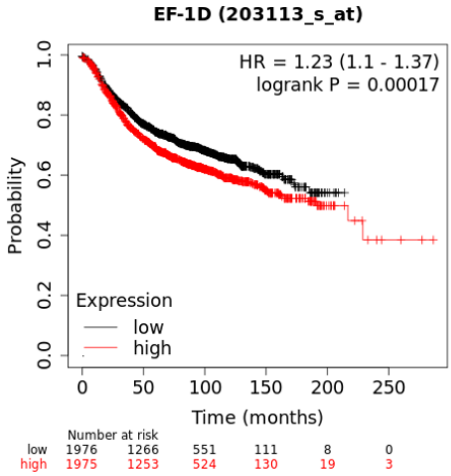

Figure 3: Kaplan-Meier survival curve showing relapse-free survival in relation to eEF1D mRNA expression levels in breast cancer

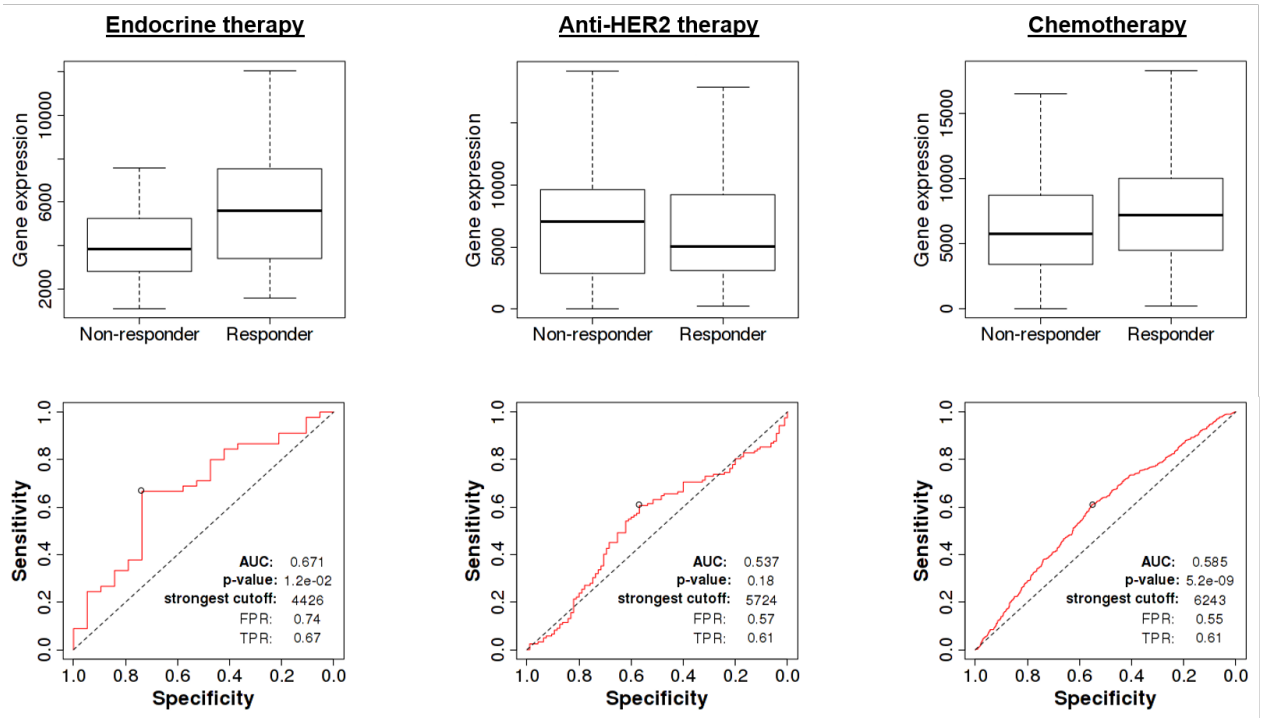

Figure 4: Prediction of response to therapy in relation to eEF1D mRNA expression levels in breast cancer (upper panels). Lower panels indicate the predictive potential of eEF1D gene as a biomarker for the respective therapy alternative

4 Discussion

Breast cancer is the most frequently encountered cancer type in women and is the leading cause of cancer-related deaths [16]. Due to its heterogenous nature with multiple subtypes, it is critically important to better understand the molecular mechanisms of its carcinogenesis and to identify prognostic and predictive biomarkers, which will aid in providing better tailored treatment options and improving survival rates.

Chromosomal aberrations such as amplification or deletion of a genomic locus, gene duplications and translocations, as well as single nucleotide variations are characteristic features of cancer. 8q24 amplification is one of the most frequently genomic alterations in breast cancer, often resulting in the overexpression of proto-oncogenes such as MYC in high grade tumors [21]. There are also several cancer-related missense mutations and miRNA clusters within the 8q24 locus and previous reports suggested that MYC overexpression might not always be the sole link between 8q24 amplifications and cancer [22]. Therefore, in this study we analyzed the effects of genomic alterations in eEF1D gene, which lies within the cancer risk associated 8q24 locus, and evaluated its prognostic and predictive significance in breast cancer.

We found that eEF1D was highly amplified in the TCGA PanCancer Atlas dataset for BRCA, while the number of patients who had homozygously deleted eEF1D was low. In line with the amplification events, we observed elevated levels of both eEF1D mRNA and protein. Most of the patients in the dataset were diagnosed with breast invasive ductal carcinoma as expected, since it accounts for the most common subtype of BRCA [23]. Histopathologically, eEF1D amplifications appeared to result in increased cellular abnormalities and structural signatures of cancer, whereas tumor samples with deletions of eEF1D had increased tubular formation. The degree of tubular formation is one of the parameters in grading breast tumors and is associated with better prognosis [24]. Taken together, our histopathological evaluations suggest a cancer-promoting role for eEF1D. Moreover, we also showed that increased expression of eEF1D at the mRNA level correlated with shorter relapse-free survival, indicating a negative association between eEF1D expression levels and the prognostic outcome.

Surgical intervention, chemo-, endocrine and anti-HER2 therapies are the main treatment options for breast cancer [25]. The treatment regime for a patient is determined based on the size, location and the molecular markers of the tumor, as well as the presence or absence of metastasis and involvement of the lymph nodes [26]. Endocrine therapy, also known as hormonal therapy, is the treatment of choice for hormone receptor (ER and PR) positive breast cancer [27]. A predictive biomarker indicates whether a patient carrying it would benefit from a specific treatment over an alternative treatment [28]. In this respect, it differs from prognostic markers that predict the risk of disease recurrence. Here in this paper, we showed that patients with elevated levels of eEF1D responded significantly better to endocrine therapy in comparison to chemotherapy or anti-HER2 therapy. Furthermore, eEF1D performed quite well as a predictive biomarker for endocrine therapy as evident by the high AUC value. Therefore, we can strongly claim that although eEF1D presents an unfavorable prognostic factor in breast cancer, it seems to have a significant predictive value in identifying patients who would respond or resist to endocrine therapy.

As a vital cellular process that regulates cellular function, protein synthesis is tightly regulated both at transcriptional and translational levels. In cancer cells, the components of the protein synthesis machinery are often mutated, differentially expressed or post-translationally modified [29]. In line with this, previous studies identified the eukaryotic elongation factor eEF1A2 and eEF1Bα as a potential oncogene and a prognostic biomarker, respectively [30,31]. This study provides further support to the previous findings in the literature implicating cancer-related roles for eEF1D and adds it to the list of elongation factors with prognostic and predictive significance.

Authors' Contributions: BBS performed the *in silico* analyses and AIK carried out the histopathological evaluations and Both authors have participated in the conception and planning of the work, interpretation of the results and drafting of the manuscript. Both authors have read and approved the final manuscript.

Availability of Data and Material: The data published in this article are based upon data generated by the TCGA Research Network (https://www.cancer.gov/tcga) and The Human Protein Atlas (https://www.proteinatlas.org). All data analyzed during this study are included in the published article.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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