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Invited Mini Review

Prognostic role of *EGR1* in breast cancer: a systematic review

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***EGR1* (early growth response 1) is dysregulated in many cancers and exhibits both tumor suppressor and promoter activities, making it an appealing target for cancer therapy. Here, we used a systematic multi-omics analysis to review the expression of EGR1 and its role in regulating clinical outcomes in breast cancer (BC). *EGR1* expression, its promoter methylation, and protein expression pattern were assessed using various publicly available tools. COSMIC-based somatic mutations and cBioPortal-based copy number alterations were analyzed, and the prognostic roles of *EGR1* in BC were determined using Prognoscan and Kaplan-Meier Plotter. We also used bc-GenEx-Miner to investigate the *EGR1* co-expression profile. *EGR1* was more often downregulated in BC tissues than in normal breast tissue, and its knockdown was positively correlated with poor survival. Low *EGR1* expression levels were also associated with increased risk of ER+, PR+, and HER2- BCs. High positive correlations were observed among *EGR1*, *DUSP1*, *FOS*, *FOSB*, *CYR61*, and *JUN* mRNA expression in BC tissue. This systematic review suggested that EGR1 expression may serve as a prognostic marker for BC patients and that clinicopathological parameters influence its prognostic utility. In addition to *EGR1*, *DUSP1*, *FOS*, *FOSB*, *CYR61*, and *JUN* can jointly be considered prognostic indicators for BC. [BMB Reports 2021; 54(10): 497-504]**

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INTRODUCTION

Breast cancer (BC) is the most commonly occurring invasive cancer in women worldwide and the second leading cause of cancer-related deaths in women after lung cancer. Although the overall methods for screening, diagnosis, and treatment of BC have improved in recent years, prognosis remains poor (1). More than one million new cases of BC are reported per year, and the risk of an individual dying from this life-threatening disease is 1/35 (2). Therefore, the identification of more effective and specific biomarkers for the prognosis of BC patients is of paramount importance. The development of BC is usually attributed to multi-gene mutations (3). Molecular targeted treatments have recently transformed the therapeutic approach for various tumors. To adopt a targeted therapy for the treatment of BC patients, it is critical to better understand the status of various molecular processes, such as gene expression and methylation of the related genes.

The early growth response 1 (*EGR1*) gene encodes a protein belonging to the early growth response (EGR) protein family, a family of zinc finger transcription factors. Various cytokines, hormones, and DNA-damaging agents can temporarily activate EGR1, and EGR1 itself functions as a transcriptional regulator (4). Moreover, EGR1 is a direct regulator of several tumor suppressors, such as transforming growth factor beta 1 (TGFβ1), tumor protein P53 (p53), and phosphatase and tensin homolog (PTEN). In addition, *EGR1* is highly overexpressed in colorectal, gastric, liver, and uterine cervical cancer, which is associated with distant metastases and poor survival (5-8). On the other hand, *EGR1* has been identified as a tumor suppressor in rhabdomyosarcoma, and it was reported that overexpression of EGR1 prevents proliferation, mobility, and anchorage-independent growth of rhabdomyosarcoma cells (9). The upregulation of *EGR1* has also been reported to arrest cell cycle progression in BC cells (10). In molecular targeted therapy approaches, this gene has been suggested as a potential target for prostate cancer (11). In patients with non-small cell lung cancer, suppressed EGR1 expression is directly associated with poor survival through attenuating PTEN expression following surgical resection (12). In another study, knockdown of EGR1 increased

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lung cancer cell proliferation by directly suppressing cytokeratin 18 (KRT18) expression (13). Suppression of EGR1 has the potential to induce the proliferation of hematopoietic stem cells in mice bone marrow (14) and prevents glioma proliferation via downregulation of *CCND1* (Cyclin D1) promoter activity (15). In addition to these roles in different types of cancers, the association of *EGR1* with diagnosis and clinical outcomes in BC patients has attracted much research attention worldwide. Loss of EGR1 expression can potentially prevent the activation of the multidrug resistance protein 1 (*MDR1*) promoter in paclitaxel-resistant MCF7 cells and thus, can regulate *MDR1* expression (16). Overexpression of *MDR1* results in multidrug resistance, which leads to failure of BC chemotherapy. Downregulation of *EGR1* is, therefore, associated with poor prognosis in BC, labeling *EGR1* as a cancer suppressor gene (16-18). A previous study (19) suggested that *EGR1* can regulate BC cell metabolism and may be a promising target to prevent endocrine resistance.

The *EGR1* gene is associated with the pathogenesis of various tumors, including breast tumors (10). However, its prognostic value in BC is controversial. Despite the reasonable volume of related research, the application of EGR1-assisted targeted therapy is still in the early stages, and the use of its expression level as a prognostic marker in BC is an area of active investigation. Therefore, in this study, we sought to investigate the roles of *EGR1* in BC. In this study, we systematically reviewed the biomarker utility and prognostic significance of *EGR1* in human BC using multiomics analysis. We comprehensively analyzed *EGR1* expression pattern, its promoter methylation status, various functions, and different prognostic impacts on BC using all currently available gene expression data. This multiomics analysis ultimately demonstrated that EGR1 expression can be adopted as a biomarker for the prognosis of BC patients.

SEARCH STRATEGY AND METHODS

We performed a PubMed and Scopus literature search until June 2021 using keywords: EGR1 and cancer/breast cancer (BC), signaling pathway, treatment, and therapeutics. In this review, we included English language articles focused on EGR1-related BC progression and prognosis, and its therapeutic applications.

EGR1 mRNA expression in various cancers was analyzed and displayed using the Oncomine platform (https://www.oncomine.org/resource/login.html; accessed February 2021) (20-23). The default threshold parameters were selected, which consisted of p-value, 1E-4; fold-change, 2; and gene ranking in the top 10%. Statistical analysis was performed using an unpaired t-test and $P < 0.05$ was considered significant. Genes co-expressed with *EGR1* were retrieved from the Oncomine database. Co-expression heatmap data for *EGR1* were downloaded from the Oncomine database.

TCGA data regarding *EGR1* mRNA expression in human BC was analyzed and displayed using the UALCAN web tool (http://ualcan.path.uab.edu/index.html; accessed July 2020) (23, 24). Statistical analysis was performed using a Student's t-test and $P < 0.05$ was considered significant.

TCGA data regarding *EGR1* mRNA expression in human BC was analyzed using UCSC Xena. The TCGA RNA-seq data of EGR1 mRNA expression was downloaded from UCSC Xena (https://xenabrowser.net/heatmap/; accessed January 2021) (25) for BC subcategories, including PAM50 subtypes, clinical subtypes, and stages. The raw data were reanalyzed and plotted by GraphPad Prism v9.0 (GraphPad, San Diego, CA, USA). Statistical analysis was performed using an unpaired t-test with Welch's corrections for two groups and one-way ANOVA for multi groups. $P < 0.05$ was considered significant.

EGR1 protein expression in BC and its normal tissue was analyzed by immunohistochemistry (IHC). The tissue images were downloaded from the human protein atlas web (https://www.proteinatlas.org/; accessed January 2021) (26, 27). The antibody (CAB019427) against EGR1was used for IHC analysis. The intensity of EGR1 expression was measured using ImageJ following Crowe *et al*.'s protocol (28), then the data were calculated and plotted using Prism 7 (GraphPad).

Median methylation level of the *EGR1* gene promoter in human BC was analyzed using TCGA (Methylation 450K) data through the TCGA Wanderer web tool (http://maplab.imppc.org/wanderer/; accessed July 2020) (29, 30). Statistical analysis was performed using an unpaired t-test with Prism 7 software (GraphPad), and $P < 0.05$ was considered significant.

The Catalog of Somatic Mutations in Cancer (COSMIC) web resource (https://cancer.sanger.ac.uk/cosmic) (31) was used to analyze EGR1 protein somatic mutations in human cancer. A pie-chart was constructed showing the percentage of different EGR1 mutation types in BC. The cBioPortal web tool (http://www.cbioportal.org/; accessed July 2020) (32, 33) was also used to analyze the frequency of mutations and their location in the EGR1 protein in BC.

Survival analysis of BC patients with high or low *EGR1* mRNA expression levels was performed using the PrognoScan database (http://dna00.bio.kyutech.ac.jp/PrognoScan/; accessed July 2020) (34) and Kaplan-Meier Plotter (http://kmplot.com/analysis/; accessed January 2021) (35). Survival plots, log-rank P-values, and hazard ratios (HRs) with 95% confidence intervals (CI) were retrieved from the online tools. A log-rank P-value < 0.05 was considered significant.

The co-expression of *EGR1*, *DUSP1*, *FOS*, *FOSB*, *CYR61*, and/or *JUN* genes was analyzed using the UCSC Xena web tool (http://xena.ucsc.edu/; accessed January 2021) (36), with the TCGA BC cohort (TCGA-BRCA). Heatmaps and regression analyses of the co-expressed genes were retrieved from the UCSC Xena tool. Pearson and Spearman correlation analyses were also performed.

Co-expression between EGR1 or other genes was analyzed and displayed using bcGenExMiner v4.1 (http://bcgenex.centregauducheau.fr/BC-GEM/GEM-Accueil.php?js=1 accessed January 2021) (37). Statistical analysis was performed using a Welch's

test with a Dunnett-Tukey-Kramer's test and $P < 0.05$ was considered significant.

EGR1 PROTEIN STUCTURE AND ITS BIOLOGICAL ROLES

The EGR1 protein contains 543 amino acids in humans, consisting of three Cysteine 2-Histidine 2 (C2H2) zinc fingers DNA-binding domains (Fig. 1A) (38). It also contains a strong activation domain, repressor domain (also known as NAB binding site), a nuclear localization domain, and a weak activation domain. Protein kinases and phosphatases controls the phosphorylation of the different EGR1 domains (39). The protein activates or represses specific genetic programs based on its "phosphorylation/acetylation pattern". The T309 and S350 sites are phosphorylated by protein kinase B (PKB, alias AKT); whereas S378, T391, and T526 sites are phosphorylated by casein kinase II (38). Depending on its post-translational modification statues, EGR1 shows various transcriptional activation or repression functions. SUMO1 can be responsible for SUMOylation of EGR1 at K272. Also, the inhibition of Egr1 transcriptional activity can be triggered by transcriptional co-repressors NGFI-A binding proteins NAB1 and NAB2 via binding to the repressor domain.

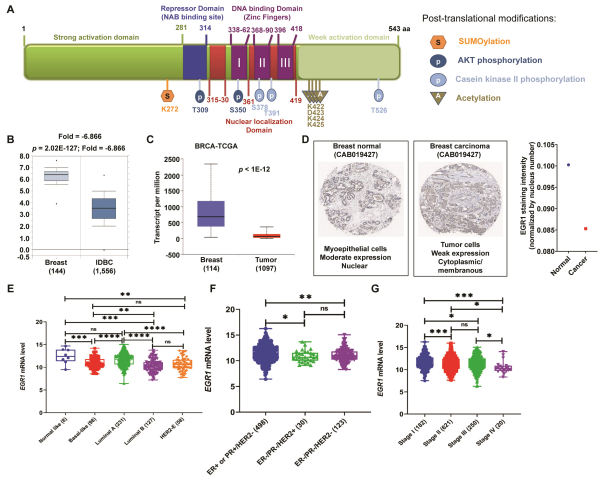

Fig. 1. Analysis of EGR1 protein structure, post-translational modification, and expression in breast cancer (BC). (A) Schematic diagram of EGR1 protein structure and post-translational modifications. (B) mRNA expression of EGR1 in normal and BC tissue (IDBC, invasive ductal breast carcinoma) was derived from Oncomine database. (C) mRNA expression of EGR1 in breast normal and cancer tissues was derived from UALCAN web using TCGA database. (D) Protein expression of EGR1 in breast normal and cancer tissues by immunohistochemistry (IHC) was derived from Human Protein Atlas web. The intensity of EGR1 expression was quantified by ImageJ and plotted by GraphPad Prism 7 software (right panel). (E-G) mRNA expression of EGR1 in BC clinicopathological subtypes was analyzed using the BRCA TCGA datasets through UCSC Xena web. Box plots showing the EGR1 mRNA expression in BC subcategories including PAM50 subtypes (E), clinical subtypes (F), and stages (G). *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

EGR1 plays a significant role in the growth, proliferation, and differentiation of various types of cells (40, 41). Although the detailed mechanisms are not yet well characterized, EGR1 plays diverse biological roles in cell signaling. High expression of EGR1 is involved in the acute phase of IL-4 transcription elevation in response to T cell receptor stimulation (40). Duclot and Kabbaj reviewed that EGR1 also regulates brain plasticity and neuropsychiatric disorders (42). Overexpression of EGR1 induced synaptic plasticity, wound repair, female reproductive capacity, and apoptosis by upregulating downstream genes (43). Several studies have shown that upregulation of EGR1 contributes to the suppression of various human cancers progression except for prostate and bladder cancers (11, 12, 15, 44, 45). In addition, a study has claimed that knockdown of EGR1 could inhibit prostate cancer invasion by attenuating IL-8 production, while another study revealed nanotechnology-based EGR1-assisted targeted therapies for preventing cancer development (46, 47). However, the prognostic significance of EGR1 varies depending upon the cancer type. For example, EGR1 is considered oncogenic in prostate cancer (48, 49), whereas it is usually regarded as a tumor suppressor in BC (16, 17). Moreover, the diverse roles of EGR1 expression in the growth and metastasis of particular cancer remain largely unknown.

EGR1 MRNA AND PROTEIN EXPRESSION IN BREAST CANCER (BC)

To investigate the expression level of *EGR1* in BC and their normal counterparts, we first determined the mRNA expression pattern of *EGR1* using oncomine database. A significant low mRNA expression levels of *EGR1* in invasive ductal breast cancer (IDBC) were found (Fig. 1B; Curtis Breast ref. (50)). To crosscheck *EGR1* mRNA expression in normal breast and BC tissues, we analyzed data from the TCGA database using the UALCAN web tool. These results were in agreement with those obtained from Oncomine-based analyses. Compared to normal tissue, *EGR1* expression levels were significant underexpressed in cancer tissue (Fig. 1C). We further examined protein expression patterns of *EGR1* in BC using immunohistochemical (IHC) staining via the Human Protein Atlas. These results also confirm the underexpression of *EGR1* at the protein levels in BC samples relative to normal breast tissue (Fig. 1D). It is worth to note that the results on Oncomine and ULCAN-driven EGR1 expression pattern in BC tissues agreed with previous study (51).

CLINICOPATHOLOGICAL RELEVANCE OF EGR1 EXPRESSION IN BREAST CANCER (BC) PATIENTS

The analysis on EGR1 transcript expression reported in the preceding section considered the entire expression data for all BC subtypes combined. In clinical practice, however, subtypes of BC may be advantageous in planning overall treatment and developing precise therapies. Here, we therefore aimed to

explore the relationship of *EGR1* mRNA expression with clinicopathological variables of BC patients.

As presented in Fig. 1E-G, we performed a number of between-class mRNA expression comparisons, including both molecular and clinical subtypes using TCGA data through the UCSC Xena web. In PAM50 molecular subtypes, the lowest level of *EGR1* expression was noticed in luminal B type BC, whereas the highest level of *EGR1* expression was seen in normal like BC. The results show that the mRNA level of *ERG1* could not significantly differentiate luminal A from normal-like BC and HER2-E from luminal B and basal-like BC. For all other cases, however, significant differences in *EGR1* expression levels among the molecular subtypes exist (Fig. 1E). In clinical subtypes, the EGR1 expression level can significantly differentiate "ER+ or PR+/HER2−"-type BC from "ER−/PR−/HER2+" and "ER−/PR−/HER2−" subtypes (Fig. 1F). As revealed from the overall staging classification, although stage II BC cannot be significantly differentiated from stage III BC in terms of mRNA expression level, significant differentiation between any two of the remaining combinations are prevailed (Fig. 1G). It can be noted that the stage IV BC showed the lowest level of *EGR1* expression compared to the other stages (Fig. 1G). Thus, the clinicopathological results altogether suggest that ER, PR, and HER2 receptors can be targeted in *EGR1*-mediated targeted therapy.

METHYLATION STATUS AND GENETIC ALTERATIONS OF EGR1 IN BREAST CANCER (BC)

Epigenetic alterations in cancers can regulate gene expression. This regulation depends on the methylation on the gene promoter regions, which subsequently regulate the gene transcription. Hypermethylation on gene promoter prevents the transcription factor binding on the promoter, which eventually inhibits the gene's transcription. It is previously reported that epigenetic alteration on gene promoter modulates the gene transcription and thus regulates carcinogenesis (52-54). Therefore, we investigated the methylation status of the *EGR1* promoter in normal breast and BC tissues using TCGA Wanderer. The *EGR1* gene promoter was found to be hypermethylated in BC in all available CpG sites, and most of the results were statistically significant (Fig. 2A). Thus, the abundance of methylation level on *the EGR1* promoter region in BC might cause the downregulation of *EGR1* mRNA expression, which was detected using the Oncomine, and UALCAN tools (Fig. 1).

We then focused on the mutations and copy number alterations (CNAs) of EGR1 in BC. Somatic cells can be mutated spontaneously throughout a person's lifetime. We analyzed somatic mutations in EGR1 in BC using COSMIC. The results of the different types of mutations are presented in Fig. 2B. Of the queried samples, 9 samples were associated with somatic mutations. Most of the somatic mutations cannot show any obvious effect, while few of them can change the key molecular functions in cancer cells (55). The major mutation

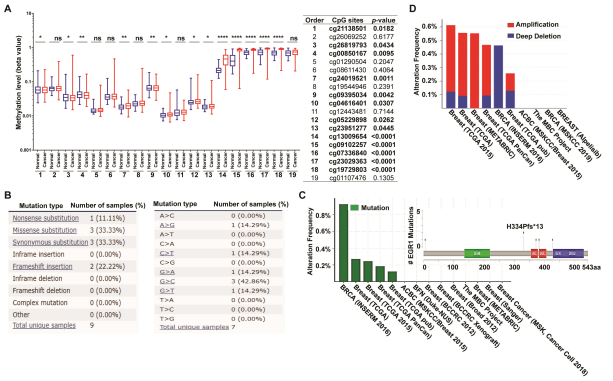

Fig. 2. Methylation status and genetic alterations of *EGR1* in BC. (A) Methylation level of the *EGR1* gene promoter in BC (TCGA Wanderer web tool). Median methylation level of the *EGR1* gene promoter in BC. The box plot comparing specific CpG sites of *EGR1* promoter methylation in normal (blue plot) and cancer tissue (red plot) was derived from the TCGA database (Methylation 450K) through the TCGA Wanderer web tool. The P values were obtained after an unpaired t-test using GraphPad Prism 7 software. (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001). (B) EGR1 mutation in human BC. Table showed the percentage of the mutation type of EGR1 in BC according to COSMIC database. (C) Alteration frequency of EGR1 mutation in BC was analyzed by using cBioPortal web. (D) Alteration frequency of EGR1 copy number in BC was analyzed by using cBioPortal web.

types were synonymous substitution, missense substitution, frameshift insertion, and nonsense substitution, with rates of 33.33%, 33.33%, 22.22%, and 11.11% of the mutant samples, respectively (Fig. 2B). Of the EGR1 mutations detected in BC tissues, 42.86% were G>C mutations (Fig. 2B). Moreover, we determined the EGR1 mutation frequency in BC using cBioPortal. These results showed that BRCA (INSERM 2016) had the most genetic alterations, accounting for approximately 1% of all samples (Fig. 2C). The mutation sites for EGR1 in BC tissues were located between amino acids 0 and 543, with a hotspot at H334Pfs*13, suggesting that mutations in *EGR1* may possess a potential role in BC progression. Moreover, we analyzed the copy number alterations (CNAs) for *EGR1* in BC. The results showed that the alterations (due to amplification and deep deletions) occurred mostly in the Breast (TCGA 2015), accounting for approximately 0.6% of all samples (Fig. 2D).

PROGNOSTIC RELEVANCE OF EGR1 EXPRESSION IN BREAST CANCER (BC) PATIENTS

We next investigated whether *EGR1* mRNA expression has any potential role on BC prognosis. To find the prognostic relevance of *EGR1* in BC, we performed survival analysis using PrognoScan and Kaplan-Meier (KM) Plotter webs. In each type of survival pattern, including overall survival (OS), relapse-free survival (RFS), distant metastasis-free survival (DMFS), and disease-specific survival, low levels of EGR1 expression

correlated with poor survival, whereas high levels of EGR1 expression were associated with high survival rates. The Prognoscan-based survival analysis showed a positive correlation between *EGR1* downregulation and poor OS rates in patients with BC (Fig. 3A). Similar correlation characteristics for EGR1 expression were found for other survival types, including DMFS, RFS, disease specific survival, and DFS (Fig. 3B-E). Next, to confirm the relevance of *EGR1* expression in BC to patient survival, we performed survival analysis using Kaplan-Meier (KM) Plotter. Like Prognoscan, KM Plotter-based survival analysis also showed that low levels of *EGR1* expression were positively correlated with poor survival for RFS and DMFS but not OS (Fig. 3F-H). Also, previous studies reported that EGR1 expression can regulate clinical outcomes in various cancers including gastric and ovarian (56, 57). Furthermore, we analyzed patient survival based on clinical subtypes. Both univariate and multivariate regression analyses confirmed that various clinicopathological parameters further regulate EGR1 expression in BC and thus the clinical outcomes of the patients. Overexpression of *EGR1* in ER+/PR+ or ER+/PR+/HER2− BC was positively correlated with high survival rates (Fig. 3I, K), whereas *EGR1* upregulation in ER−/PR− or ER−/PR−/HER2+ BC was associated with poor survival (Fig. 3J, L). The ER−/PR−/HER2− BC patient was not shown any significant difference in patient survival (Fig. 3M). The opposite outcomes of BC with *EGR1* expression in terms of clinical subtypes (ER/PR/HER2 status) may be explained as follows. From clinicopathological studies, we observed that individual PR+, ER+, and HER2− BC tissues showed high levels of *EGR1* expression, which might intuitively associate with better clinical outcomes in BC patients. In contrast, high *EGR1* expression in PR−/ER−/HER2+ type BC should, therefore, naturally be related to poor outcomes. The relationship between EGR1 expression and translational clinical relevance is further highlighted by meta-analysis (Fig. 3N-Q) using KM Plotter. Hazard ratio (HR) of RFS and DMFS in GSE20685 were significantly higher than 1, showing that elevated EGR1 expression in BC is correlated with poor clinical outcomes, while HR of RFS in GSE16391, GSE1456, GSE17705 were significantly lower than 1, showing that attenuated EGR1 expression in BC is correlated with poor clinical outcomes (Fig. 3O, P). These findings suggested that various clinicopathological parameters in general and ER, PR, and HER2 receptor status in particular, should be considered when designing EGR1-mediated targeted therapy for BC patients.

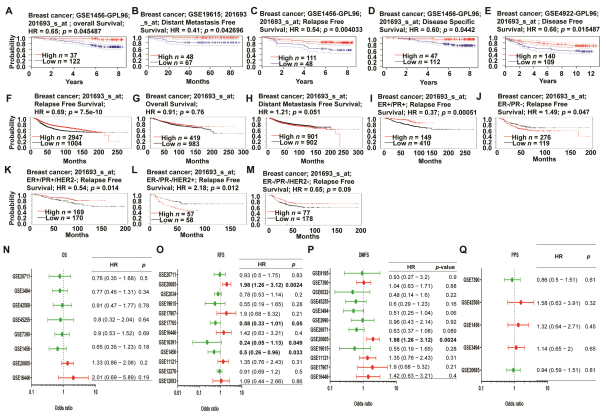

Fig. 3. Relationship between EGR1 mRNA expression and clinical outcomes in BC patients (PrognoScan and Kaplan Meier plotter Database). (A-E) The survival curve comparing the patient with high (red) and low (blue) expression of EGR1 (probe: 201693_s_at) was plotted from PrognoScan database in BC patients. (F-M) The survival curve comparing the patient with high (red) and low (blue) expression of EGR1 (probe: 201693_s_at) was plotted from Kaplan Meier plotter in BC patients. The threshold of cox P-value < 0.05. Meta-Analysis of Studies of BC studies with EGR1 mRNA expression. Forest plots of GEO datasets evaluating association of EGR1 mRNA expression with OS (N), RFS (O), DMFS (P), and PPS (Q) in BC. Hazard ratio (HR) with 95% confidential interval (CI) and *p*-value were labeled in the right column of each forest plot.

EGR1 AND CO-EXPRESSED GENES AND THEIR ASSOCIATION IN BREAST CANCER (BC) PROGNOSIS

As *EGR1* expression contributes to BC progression and prognosis, we further aimed to find the possible underlying signaling mechanism involved in *EGR1*-mediated BC progression and prognosis. For that, we first used the Oncomine platform to analyze the co-expression pattern of *EGR1* with its correlated genes in BC. In Fig. 4A, we present the top 20 genes (total count 17), ranked based on correlation coefficient values, that correlated with *EGR1*, after analyzing 53 BC and 140 normal breast samples. Based on a threshold correlation coefficient of around 0.75, the highly correlated genes were *DUSP1*, *FOS*, *FOSB*, *CYR61*, and *JUN*. To confirm the co-expression status of *EGR1* with the correlated genes, we also performed a correlation heatmap and various regression analyses. The heat maps of *EGR1*, *DUSP1*, *FOS*, *FOSB*, *CYR61*, and *JUN* showed similar expression patterns across each PAM50 BC subtype, including HER2+, luminal B, basal-like, and luminal A (TCGA data; Fig. 4B), thus supporting the Oncomine result showing that *DUSP1*, *FOS*, *FOSB*, *CYR61*, and *JUN* were highly co-expressed with EGR1 (Fig. 4A). We plotted scatter diagrams for *DUSP1* vs. *EGR1* and *FOS* vs. *EGR1* expression using UCSC Xena (Fig. 4C, D). We constructed a correlation matrix of the expression of EGR1 and the five most highly correlated genes by performing data mining in bc-GenExMiner 4.0 that includes DNA microarrays and RNA-seq data. The results showed that all the cross-correlation coefficients between any pair of genes selected from the possible combinations were highly positive (Fig. 4E). Finally, bc-GenExMiner 4.0-based regression analysis further confirmed the positive correlation of *EGR1* vs. *DUSP1* and *EGR1* vs. *FOS* mRNA expression (Fig. 4F, G). In fact, it has been reported that *DUSP1* regulates the epithelial-to-mesenchymal transition (EMT) process, affecting various signaling pathways involved in BC, such as wnt, notch, and

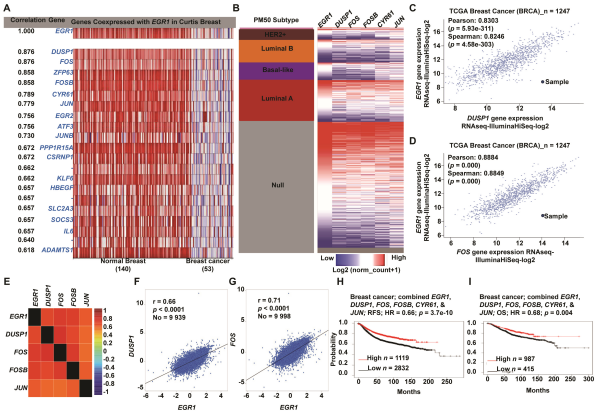

Fig. 4. EGR1 mRNA expression is correlated to DUSP1, FOS, FOSB, CYR61, and JUN mRNA expression in BC. (A) Top 20 genes positively correlated with EGR1 mRNA expression based on 2, 136 BC samples in Curtis Breast (PMID: 22522925). Analysis was performed using Oncomine database. (B) The heat map of EGR1, DUSP1, FOSB, CYR61, and JUN mRNA expression across PAM50 BC subtypes in TCGA database. Data was analyzed using UCSC Xena (http://xena.ucsc.edu/). (C, D) Regression analysis showed that EGR1, DUSP1, and FOS had positively high correlation coefficients. Data was analyzed using UCSC Xena (http://xena.ucsc.edu/). (E) Data mining in bc-GenExMiner 4.0 confirmed the positive correlation between EGR1, DUSP1, FOSB, and JUN mRNA expression across DNA microarray data. (F, G) Regression analysis confirmed that EGR1, DUSP1, and FOS had positively high correlation coefficients across DNA microarray data. Data was analyzed using bc-GenExMiner 4.0 web. (H, I) The survival curve comparing the patient with high (red) and low (blue) expression of *EGR1*, *DUSP1*, *FOS*, *FOSB*, *CYR61*, and *JUN* was plotted from Kaplan Meier plotter in BC patients. The threshold of cox P-value < 0.05.

mitogen-activated protein kinase (MAPK) pathways (58). A significant reduction in *DUSP1* mRNA expression has been reported in BC tissue compared with that in normal breast tissue (59). Another study reported that *FOS* expression is associated with intracellular signaling events affecting BC cell growth (60, 61) and the overexpression of *FOS* has been associated with improved clinical outcome (62). This association between higher *FOS* expression and improved clinical outcome was also seen in our analysis, as we showed that the prognostic significance of *EGR1* co-expression with *DUSP1* and *FOS*. It is worth noting that *DUSP1* has previously been reported to be overexpressed in BC (63, 64). *FOS*, *FOSB*, and *JUN* expression has also been associated with BC and correlates with various clinicopathological parameters (65-67). Likewise, a number of researchers had reported that increased expression of *CYR61* is associated with BC progression (68, 69). Finally, we also analyzed the prognostic relevance of the co-expression of EGR1 with the set of highly correlated genes. High levels of co-expression of these genes were associated with a good prognosis of both OS and RFS (Fig. 4H, I), suggesting that the co-expression of *EGR1* with *DUSP1*, *FOS*, *FOSB*, *CYR61*, and *JUN* can also regulate the clinical outcomes of patients with BC.

CONCLUDING REMARKS

In this study, we used various web-based bioinformatics tools to perform a multiomics analysis of *EGR1* mRNA expression, promoter methylation, somatic mutation, and clinical outcome data to investigate the impact of *EGR1* on human breast cancer (BC). Based on *EGR1* expression, promoter methylation, protein expression pattern, and prognosis status, our analysis showed that this gene was more often under-expressed in BC tissues especially in Ductal breast carcinoma, invasive ductal breast carcinoma, and medullary breast carcinoma subtypes and its downregulation was positively correlated with poorer prognosis. Moreover, different clinicopathological parameters, such as ER, PR, and HER2 status play important roles in regulating the expression pattern of *EGR1* in patients with BC, which eventually modulates patient survival. Furthermore, we found that *EGR1* expression was highly positively correlated with *DUSP1*, *FOS*, *FOSB*, *CYR61*, and *JUN* expression. The results of this multiomics analysis suggested that *EGR1* can be targeted for the treatment of patients with BC and its co-expression with *DUSP1*, *FOS*, *FOSB*, *CYR61*, and *JUN* can be considered as a prognostic indicator. The present findings also reveal the significance of *EGR1* expression and possible *EGR1*-related pathways in BC progression.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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CONFLICTS OF INTEREST

The authors have no conflicting interests. The sponsors had no role in the design, execution, interpretation, or writing of the study.

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