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A multi-omics approach to reveal the key evidence of *GDF10* as a novel therapeutic biomarker for breast cancer

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ABSTRACT

Breast cancer (BC) is the most common type of invasive cancer diagnosed in women. It is the second leading cause of death from cancer and the utmost important medical concern women face today. Growth differentiation factor 10 (GDF10) is a member of the transforming growth factor β (TGF-β) superfamily and has been found to play a central role during the growth and differentiation of developing tissues. Recent studies have demonstrated the linkage between GDF10 and different types of cancer. But the relation of GDF10 expression in developing BC has not been established with concrete evidence. Therefore, we performed a multi-omics analysis to evaluate the potentiality of GDF10 as a therapeutic biomarker for human BC. We analyzed the mRNA expression patterns of GDF10 in BC subtypes using Oncomine, GEPIA2, immunohistochemistry, and UALCAN databases. Resultant data obtained from the analysis has provided clear evidence to the downregulation of GDF10 expression in BC subtypes. Additionally, three subsequent missense mutations were identified with a frequency of 0.62%–2.95% copy number alterations in the GDF10 protein sequence by analyzing 16 BCE studies from the cBioPortal database. Furthermore, the Kaplan-Meier plots revealed a positive correlation between the downregulation of GDF10 and the lower survival rate of the BC patients. The co-expressed genes profile of GDF10 was also associated with BC development. ABCA8 has been identified as the most positively co-expressed gene, which was confirmed by correlation analysis using bc-GenExMiner and UCSC Xena server. We also determined different cancer progression pathways mediated by GDF10 and its co-expressed genes by utilizing the Enrichr database. Cumulatively, the outcome data conclude that the down expression of GDF10 is associated with BC progression and patient's survival, which may serve as a therapeutic biomarker for treating BC.

1. Introduction

Cancer is a genetic or acquired disorder that is driven by the transition of a normal cell to a malignant tumor by changing the hereditary material of cells. It is the second leading cause of death globally, which accounts for about one in six of all deaths. The World Health Organization (WHO) estimates 18.1 million cancer cases and 9.6 million deaths worldwide in 2018 [1,2]. Breast cancer is one of the most prevalent forms of cancer in which cells in the breast grow out of control. In 2018, an estimation of 2.09 million new cases and 6,26,679 deaths occurred due to breast cancer. The survival rate of breast cancer patients has increased with early detection, diagnosis, and significant advances in treatment and palliative care [3]. However, breast cancer still causes a large number of deaths consisting of around 15% cancer deaths among women. Identification of a therapeutic target can be a promising approach to reduce this clinical threat to survival [4,5].

Altered gene expression, is the result of gene mutations have frequently reported as a leading cause of breast cancer development. Moreover, the properties of these differentially expressed genes (DEGs) represent the clinical characteristics of breast cancer that are related to

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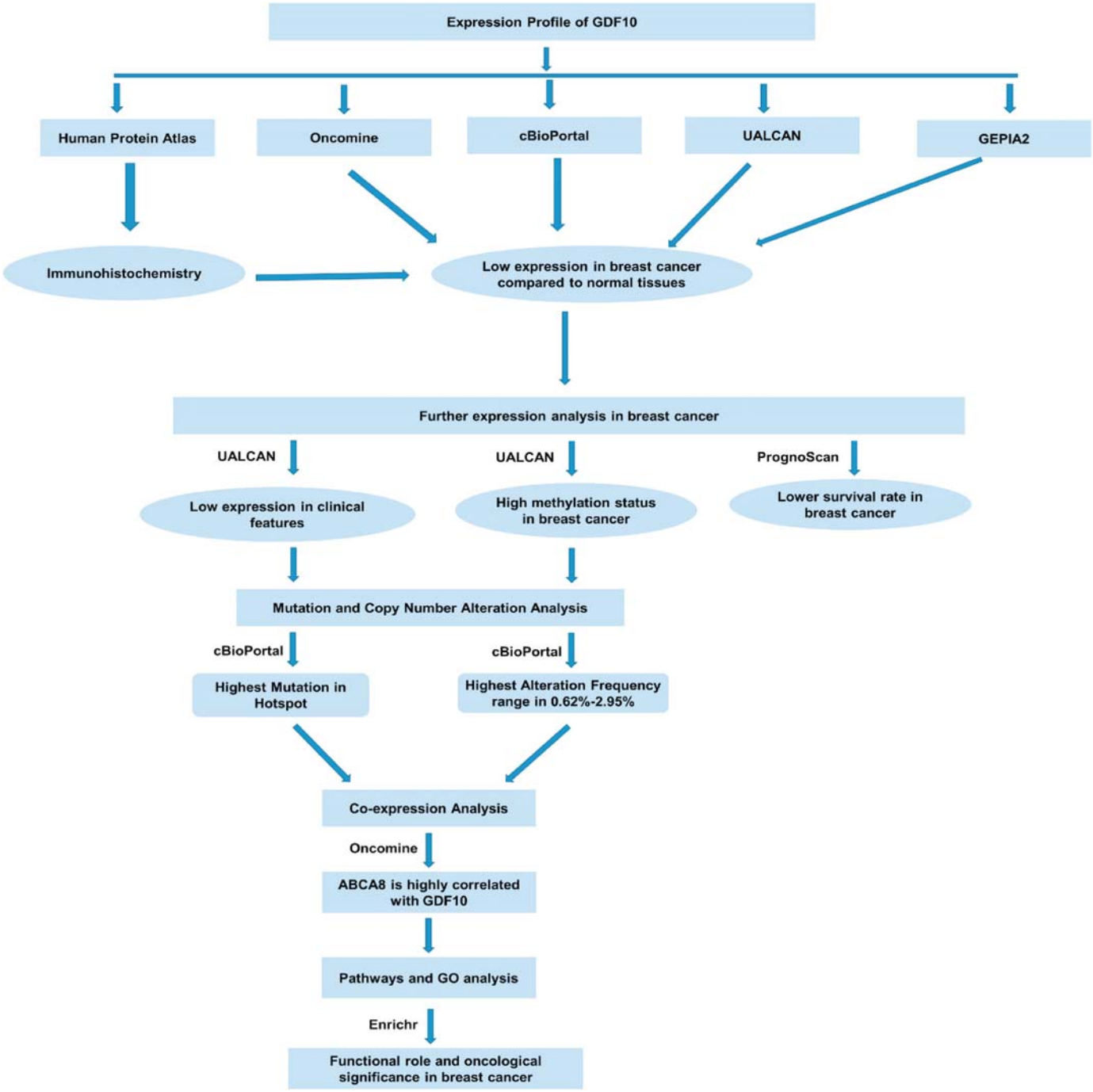

Fig. 1. A schematic diagram representing the overall workflow of the current study.

the patient's survival. For these reasons, DEGs correlated with a patient's prognosis in breast cancer can be utilized as the biomarkers for early stage detection of the carcinogenic condition as well as for more effective treatment [6–8]. Importantly, understanding the significant mechanisms of cancer progression associated with DEGs is a fundamental requirement to certify the gene as a therapeutic target [9–15].

Growth differentiating factor 10 (GDF10) is a prominent member of the transforming growth factor β (TGF-β) superfamily. GDF10 regulates the cell proliferation and differentiation by utilizing the TGF-β signaling pathway. GDF10 interacts with SMAD7 to regulate the TGF-β signaling pathway. Malfunction of the GDF10 regulated through the TGF-β pathway is associated with cancer metastasis, and defective TGFβ cytostatic responsiveness in immune cells can lead to different types of cancers [7]. Several studies confirmed the role of the TGF- β signaling pathway in promoting breast cancer due to the mutated expression of GDF10 [16–18]. Researchers have also predicted the tumor-suppressive role of GDF10 through observing the higher cell proliferation and tumorigenesis experimentally, by knocking down GDF10 in murine and human cell lines [19–22]. A few studies also pointed out the potential inhibition of GDF10 in several niches of breast cancer, but do not reflect the whole scenario of the tumor-suppressive role of GDF10 in breast cancer to confirm a probable link between them [23]. Therefore, we investigated the expression pattern of GDF10 in breast cancer from a multi-omics perspective to confirm the predicted link and establish its clinical significance as a therapeutic biomarker for diagnosis, prognosis, and drug research.

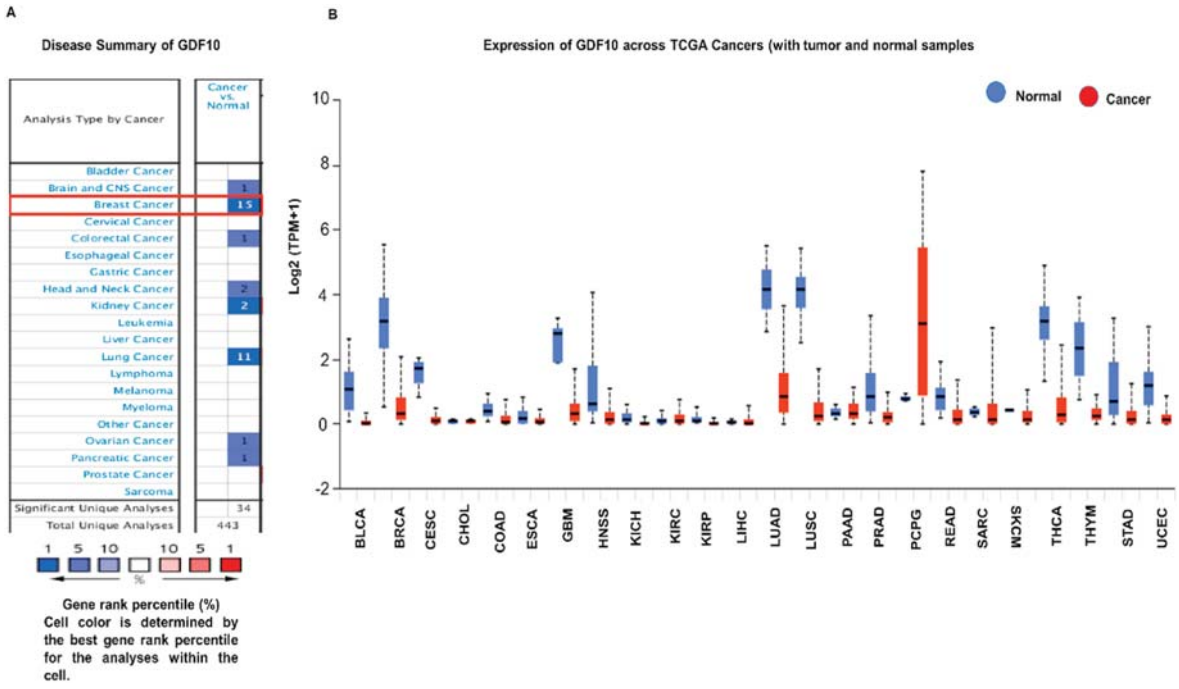

Fig. 2. GDF10 mRNA expression in different cancer types: (A) mRNA upregulation (Red) and downregulation (Blue) of GDF10 in different types of cancer. GDF10 is downregulated in different cancer types including breast cancer, which is indicated in blue color. (B) Expression of GDF10 across various TCGA cancer data with tumor (red) and normal samples (Blue) was depicted as boxplots. The values between upper and lower quartile inside the box and dashed lines indicated the upper and lower limit of average expression. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2. Methods and materialsmaterials-methods

A flow chart representing the overall procedure for the target identification and analysis has been illustrated in Fig.1.

2.1. GDF10 mRNA expression in different human cancers

The mRNA expression level of GDF10 in different carcinogenic conditions was analyzed by using the Oncomine (https://www.oncomine.org/) database with the default threshold parameters. Oncomine is a web-based data-mining platform used to analyze the multi-omics expression of genes associated with cancer development [24,25]. The pan-cancer analysis of GDF10 was observed through the UALCAN ((http://ualcan.path.uab.edu/) database. UALCAN is a public-accessible database that is used to characterize the differentially expressed genes (DEGs) in terms of multi-cancer progression based on the TCGA dataset [26].

2.2. GDF10 expression analysis in BC and normal tissue

The mRNA expression analysis of GDF10 in breast cancer has been performed by using the Oncomine database, Gene Expression Profiling Interactive Analysis 2 (GEPIA2) tools and the UALCAN web server [26,27]. A threshold restriction was applied for obtaining the significant result with p-value ≤ 0.05. The Oncomine analysis compares the normal tissue-specific expression of GDF10 with the cancer tissues. The variant expression of GDF10 in carcinogenic conditions was also investigated by observing the immunohistochemistry of the breast cancer cells retrieved from the Human Protein Atlas (HPA) database. GDF10 mRNA expression was also analyzed based on the clinicopathological parameters of breast cancer prognosis by using the TCGA data of the UALCAN database.

2.3. GDF10 promoter methylation analysis in BC based on clinical characteristics

The TCGA data responding to the GDF10 promoter methylation in breast cancer was generated by using the UALCAN database [26]. The methylation status of GDF10 was analyzed based on the multi-dimensional clinicopathological characteristics of breast cancer patients. Only statistically significant results were considered for the methylation analysis.

2.4. Genomic mutation and copy number alteration (CNAs) in GDF10 protein sequence

The genomic mutations and copy number alteration (CNAs) in the GDF10 protein sequence were determined by studying 16 different breast cancer studies from the cBioPortal (https://www.cbioportal.org/) server [28,29]. The mutation analysis estimates the location, frequency, and type of genetic changes in the amino acid residues.

2.5. GDF10 expression in comparison to survival of breast cancer patient

Survival analysis has been observed using the Kaplan-Meier Plotter server to identify the clinical impact of GDF10 expression in breast cancer progression. The Kaplan-Meier plotter is a publicly accessible web platform that performs a meta-analysis based on multi-omics datasets [30,31]. To analyze the prognostic value, the gene expression level was divided into the upper and lower quartile. The resulting survival plots depicted the correlation of GDF10 mRNA expression level with the survival prognosis of breast cancer patients.

2.6. Analysis of genes Co-Expressed with GDF10

The genes positively correlated with GDF10 were profiled by using the Oncomine database [24,25]. To identify genes with shared functions and similar expression patterns they were chosen as positively

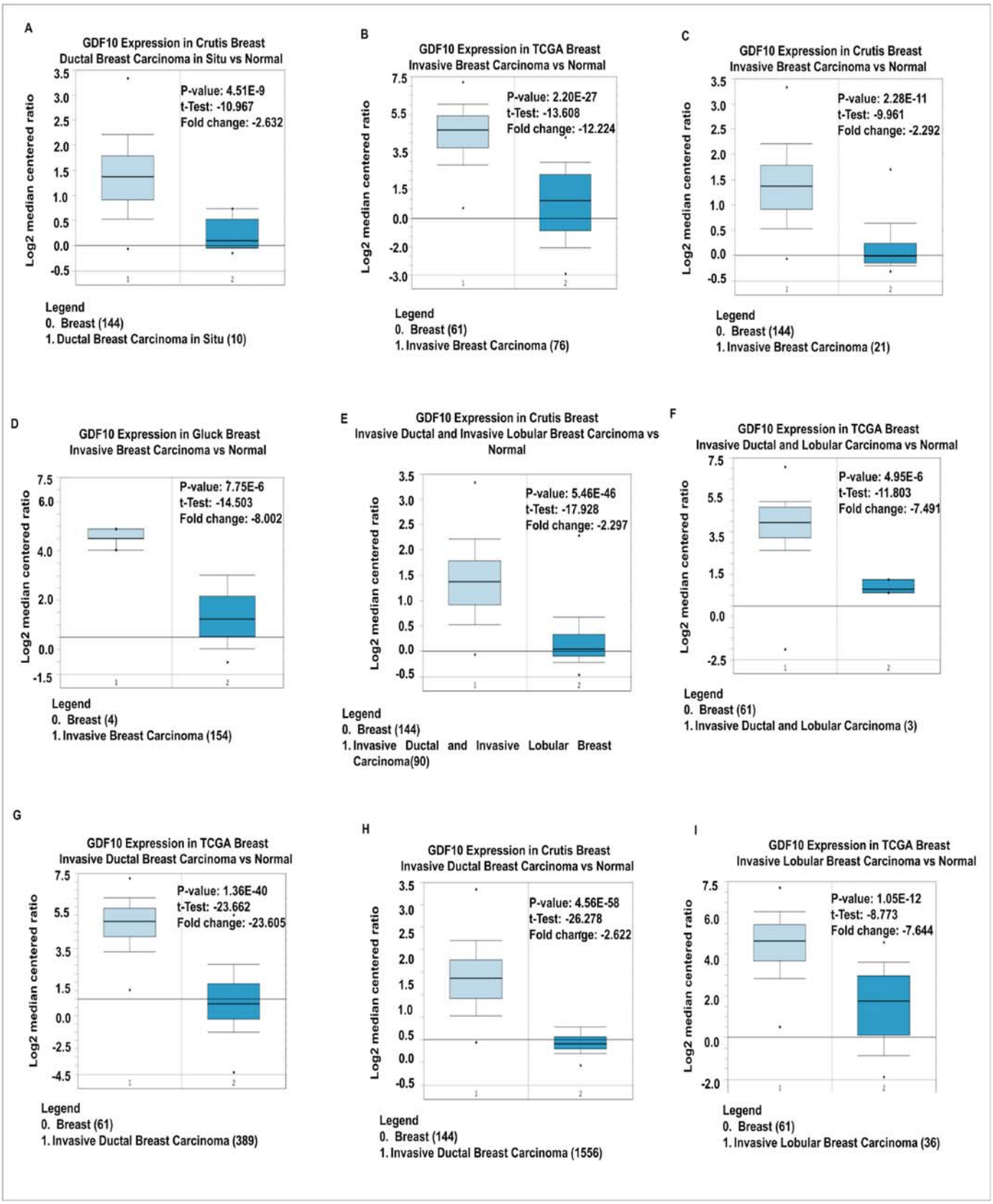

Fig. 3. Comparison of the expression pattern of GDF10 between breast cancer tissue and normal tissue. (A–O) Downregulated GDF10 expression in breast cancer. The boxplots represented GDF10 expression specific fold change. (P) The decreased expression of GDF10 in breast cancer compared to normal tissue was determined by using GEPIA 2 as depicted in boxplots (* means $p \leq 0.05$). (Q–R) Using the immunohistochemistry data, representative protein expression of GDF10 in normal tissue (glandular cell) and breast cancer (tumor cell).

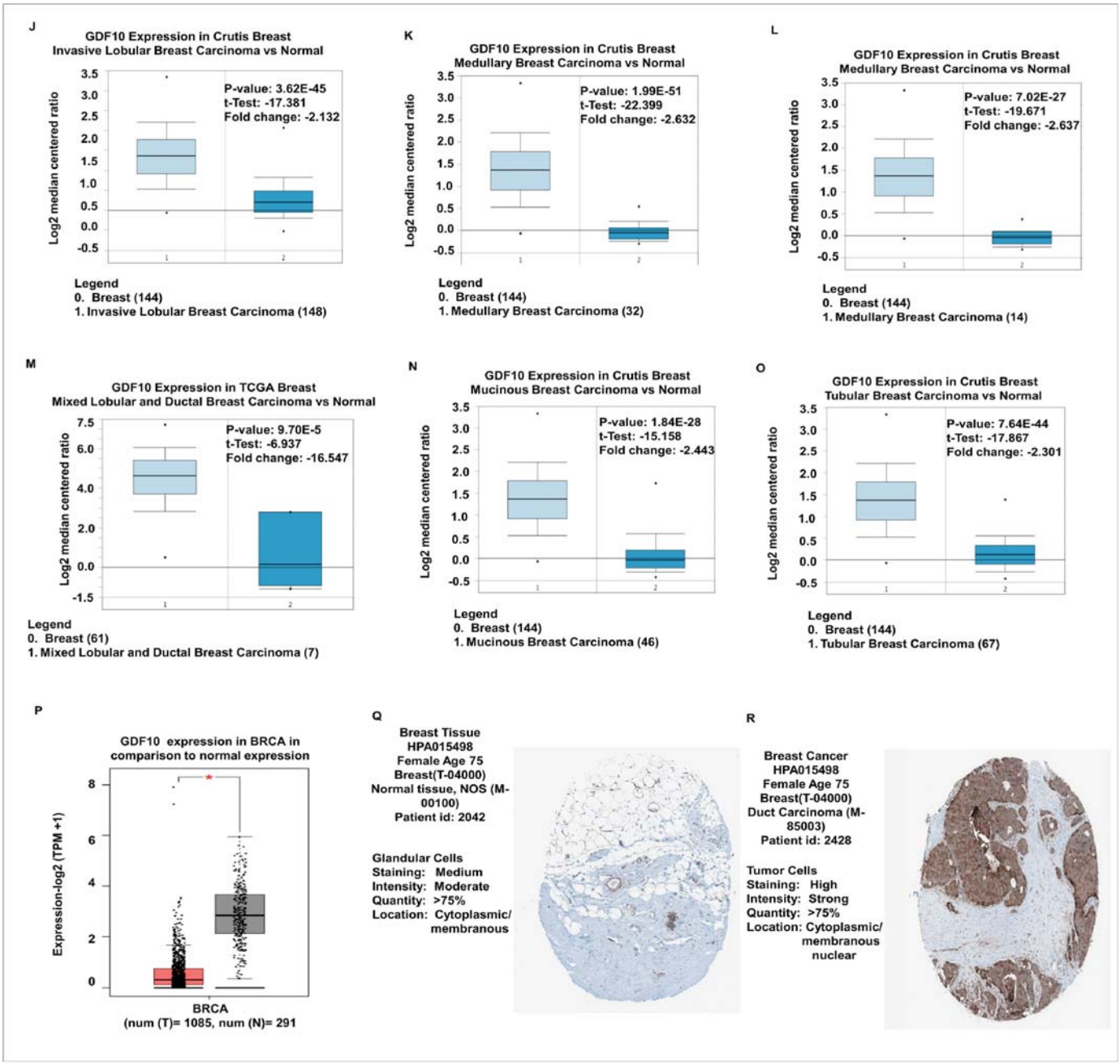

Fig. 3. (*continued*).

correlated genes with GDF10. Through this analysis, ABCA8 was found as the topmost co-expressed gene of GDF10. Therefore, the co-expression correlation between the GDF10 and ABCA8 was further visualized graphically by using UCSC Xena web [32].

2.7. Identification of genes Co-expressed with GDF10 and their signaling pathways

Co-expressed genes of GDF10 were retrieved from the Oncomine server. The identified co-expressed genes were utilized for determining the signaling pathways related to GDF10. This analysis has done by using the Enrichr web server. Enrichr consists of a large set of gene libraries for interpreting signaling pathways through diverse gene-specific experiments [33].

3. Results

3.1. GDF10 mRNA expression in different human cancers

To determine the variation of GDF10 expression in different carcinogenic conditions, we investigated the expression pattern of GDF10 in multiple cancer studies compared to normal tissues. This comparative analysis found downregulation of GDF10 in eight different cancer types, namely breast, brain and CNS, colorectal, head and neck, kidney, lung, ovarian, and pancreatic cancer. Interestingly, the highest number of significant unique analysis referring to the down expression of GDF10 related to breast cancer (Fig. 2A). After that, we investigated the variant expression level of GDF10 in 24 multi-cancer studies by using the UALCAN database. Here, we found that GDF10 is downregulated in 19 different cancer types and upregulated in 5 cancer types (Fig. 2B).

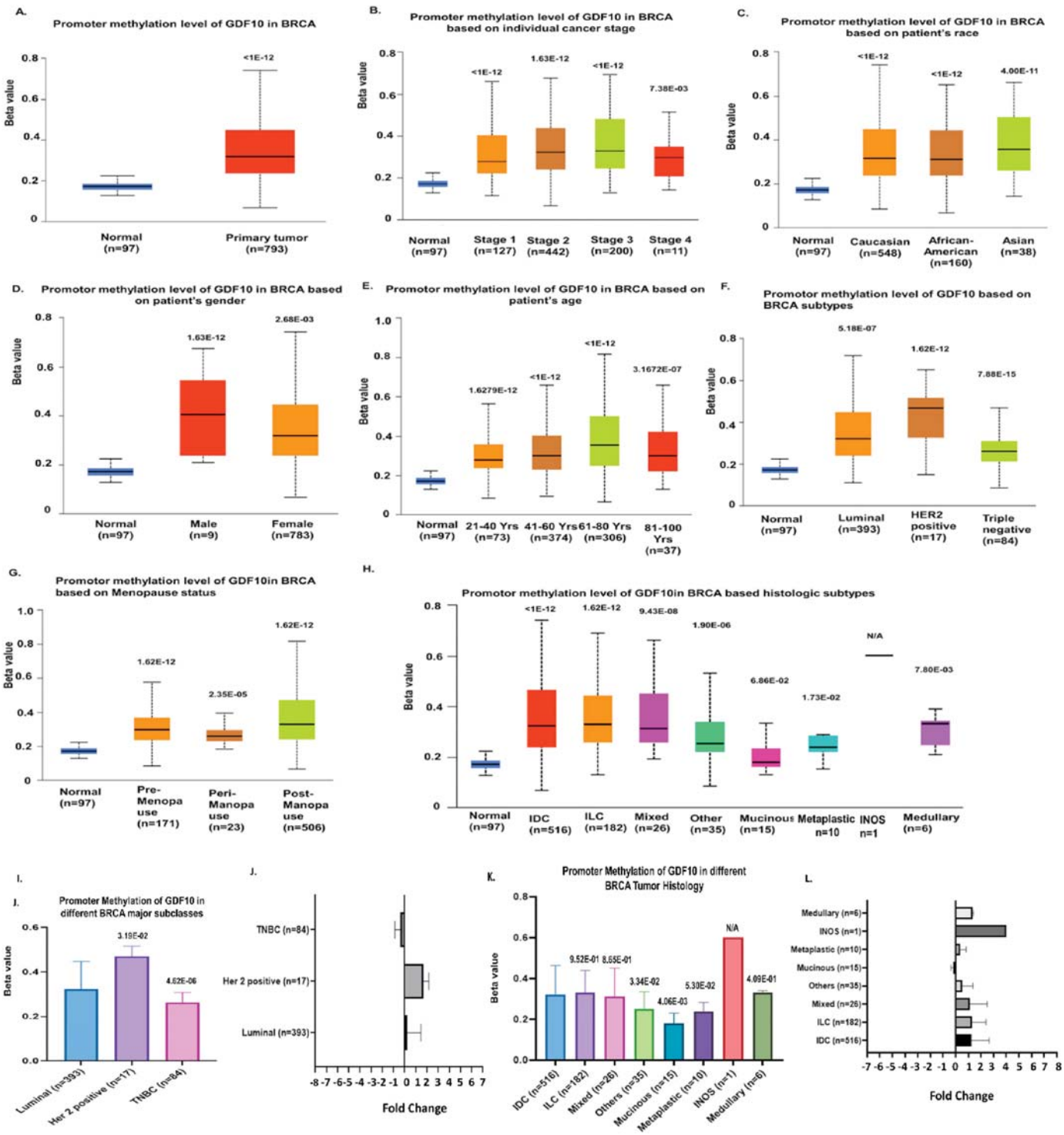

Fig. 4. The promoter methylation level of GDF10 in different clinicopathological condition was analyzed by using the TCGA dataset. The analysis compared to the methylation level of GDF10 was done based on (A) sample type, (B) stages, (C), race, (D), gender (E) age, (F) Cancer subclass, (G) Menopause Status, (H), Histologic Subtype. Then, the promoter methylation of GDF10 was compared among (I) Cancer subclasses (K) Tumor histology based on the median value of expression level (with 95% CI). The fold changes of methylation were depicted based on (J) Cancer subclasses and (L) Tumor histology.

3.2. mRNA and protein expression of GDF10 in breast cancer

To understand the GDF10 expression in multi-variant subtypes of breast cancer, we examined each of the subtypes by using the Oncomine database. Here, we differentiated the GDF10 expression in breast cancer subtypes by comparing the expression level of GDF10 in the normal condition, with medullary carcinoma, invasive breast carcinoma, tubular breast carcinoma, mucinous breast carcinoma, invasive ductal breast carcinoma, invasive ductal and lobular carcinoma, invasive lobular breast carcinoma, and mixed lobular and ductal breast carcinoma. We found a significant downregulation of GDF10 in each of the breast cancer subtypes (Fig. 3A-3O). By using the GEPIA2 database, we found a similar kind of result representing the downregulation of GDF10 expression in breast cancer (Figure 3P). Next, we observed the nature of

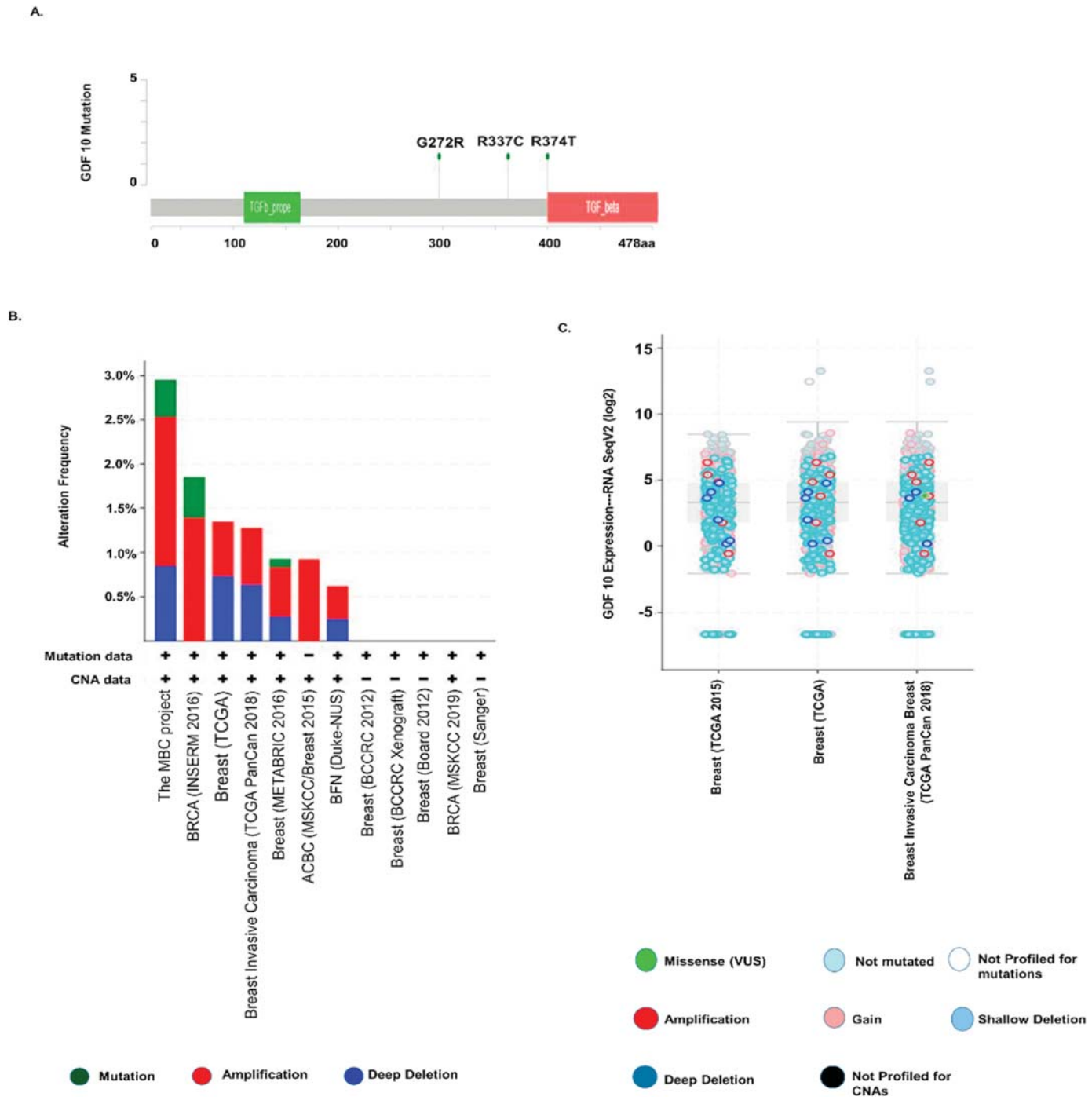

Fig. 5. Genomic mutation determination of GDF10 in breast cancer (A). Lollypop plots show missense type mutation in GDF10 (B). The alteration frequency of CNAs in GDF10 is presented as a bar chart where the green blocks indicate mutation, red blocks indicate amplification, and blue blocks indicate deep deletion type CNAs (C). Analysis of the mRNA expression of variant types of CNAs in GDF10 based on TCGA databases. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

GDF10 protein expression in normal tissue (glandular Cell) and breast cancer (tumor Cell) tissue using the data of immunohistochemistry archived from the Human Protein Atlas Project. Here, we found a medium level of staining signal in the normal glandular cells, where the staining level of tumor cell expression was higher. Additionally, the intensity signal of glandular cells was at a moderate level. But in case of tumor cells, we found a strong intensity signal (Fig. 3Q–R). The data corresponding to the expression of GDF10 in breast cancer subtypes was listed in **Supplementary Table 1**.

3.3. GDF10 expression based on clinicopathological characteristics of breast cancer

We examined the fluctuation of GDF10 gene expression based on various clinicopathological characteristics of breast cancer patients. We found significant decrease in GDF10 expression in all clinicopathological cases (Supplementary Fig. 1A). For individual cancer stages, we obtained the lowest level of GDF10 expression at stage 2 (Supplementary Fig. 1B). But among all of the stages, the most upregulated expression of GDF10 was found in stage 1. In case of the patient's race,

GDF10 expression was enormously decreased for Caucasians (Supplementary Fig. 1C). In terms of gender, we found a more down-regulated expression of GDF10 for females in comparison to male patients (Supplementary Fig. 1D). Following the age groups, we observed most decreased expression of GDF10 for 81-100-year old patients. On the other hand, age group of 21–40 years showed the highest level of GDF10 expression among the different age groups (Supplementary Fig. 1E). By analyzing the expression of GDF10 in different cancer subclasses, we identified the triple-negative subclass as the most suppressive type (Supplementary Fig. 1F). Meanwhile, the luminal subclass was found as the most dominant one among the tumor histological subgroups (Supplementary Fig. 1I). By investigating the menopause status, we found that GDF10 is mostly down expressed in post-menopause breast cancer patients (Supplementary Fig. 1G). Lastly, we went through the GDF10 expression analysis based on histological subtypes. Here, we found that expression of GDF10 was mostly decreased in IDC (Supplementary Fig. 1H). Additionally, we also observed that GDF10 was most dominantly expressed in ILC (Supplementary Fig. 1K). The graphs of fold changes demonstrated the comparative variation of GDF10 expression in between major breast cancer subclasses and tumor histology (Supplementary Fig. 4J, 4L). All of these TCGA data of GDF10 expression corresponding to the different clinicopathological status of breast cancer patients were listed in Supplementary Table 2.

3.4. Promoter methylation level analysis of GDF10 in breast cancer based on various clinicopathological parameters

Next, we investigated different promoter methylation levels of GDF10 expression based on unique clinicopathological parameters. For sample types, we observed upregulation in the promoter methylation level of GDF10 in case of primary tumors compared to the normal tissues (Fig. 4A). In terms of individual cancer stages, we found the highest level of methylation for GDF10 at stage 3. On the counterparts, the lowest level of methylation for GDF10 was found at stage 4 compared to other cancer stages (Fig. 4B). After that, we got the most increased level of GDF10 methylation status for Asian patients whereas the most decreased level was found for African American patients compared to all other races (Fig. 4C). Then, we found more upregulated promoter methylation of GDF10 for female patients versus males (Fig. 4D). Considering different age groups, we experienced the most elevated level of promoter methylation for 61-80-year old patients. On the other hand, we found the most downregulated promoter methylation level of GDF10 for 21-40-year old patients (Fig. 4E). Based on breast cancer subclasses, we explored the most increased promoter methylation level of GDF10 for Her 2-positive type (Fig. 4F). Meanwhile, the most decreased methylation level of GDF10 was found for the triple-negative breast cancer among the carcinogenic subtypes (Fig. 4I). In terms of menopause status, we experienced the highest methylation level of GDF10 at the post-menopause period (Fig. 4G). According to histological subtypes, we found the most upregulated promoter methylation level of GDF10 in ILC (Fig. 4H). In contrast, we observed most down expressed methylation levels of GDF10 for the mucinous type, comparing with all other histological subtypes (Fig. 4K). The fold changes represented the comparative variation of the GDF10 expression in between major BC subclasses and tumor histology (Fig. 4J,L). Overall, these data of variant GDF10 promoter methylation level according to different clinicopathological parameters of breast cancer patients were listed in Supplementary Table 3.

3.5. GDF10 mutation and CNA analysis associated with breast cancer development

We investigated the genomic mutations and copy number alterations (CNAs) of the GDF10 protein sequence associated with breast cancer. Here, we found three missense type of mutations on 478 amino acid long human GDF10 protein. The whole protein sequence of GDF10 includes two domains, namely, TGF- β propeptides and TGF- β like domain. Interestingly, one of the mutations was found in the TGF- β like domain (Fig. 5A). Then, we observed different types of copy number alterations at a frequency ranging between 0.62% and 2.95%. The bar graphs were constructed based on multiple studies of breast cancer where the highest frequency rate (2.95%) of alterations was found in the MBC project. The TCGA data of breast cancer also provided a higher level of alteration frequency (1.35%). Among these studies, amplification was found as the most common type of alteration (Fig. 5B). We observed the dominance of shallow deletion type CNAs over any other types of alterations. However, most upregulated expression of GDF10 was found for the gain type alteration (Fig. 5C). The datasets associated with the mutations and protein changes were listed in Table 1.

Table 1
List of mutations identified in the GDF10 protein sequence from multiple breast cancer studies.

Cancer Dataset	Sample Size	Protein Change	Mutation Type	Sample ID
MBC (INSERM PLoS Med, 2016)	216	R337C	Missense	MBC_95
MBC provisional, February 2020)	237	G272R	Missense	MBC-MBCProject_MYuoCmI7-Tumor-SM-DL4R7
BIC (TCGA PanCancer Atlas, 2016)	1034	R374T	Missense	TCGA-D8-A73U01

3.6. Correlation analysis between GDF10 expression and survival of cancer patients

Despite the functional characterization, the role of GDF10 in the clinical prognosis of breast cancer was needed to be clarified. First, we found lower GDF10 expression is correlated with the lower survival rate based on the basal-intrinsic subtype of relapse-free survival (Fig. 6A). According to the luminal A-intrinsic subtype of relapse-free survival, we determined a slightly lower survival rate was correlated with lower expression of GDF10 rather than the higher expression of GDF10 (Fig. 6B). After that, we experienced a similar result representing the positive linkage of the lower expression of GDF10 with lower survival probability when the meta-analysis was compared to higher expression of GDF10 based on grade 2 relapse-free survival (Fig. 6C). In addition, we observed that a breast cancer patient's lower clinical prognosis rate is significantly related to decreased expression of GDF10, countering the increased GDF10 expression which analysis is based on lymph node-positive status of relapse-free survival (Fig. 6D). Next, we investigated a similar type of prognostic probability report interconnected with GDF10 expression in case of luminal androgen receptor of relapse-free survival which represents that the lower prognostic probability of breast cancer patients is incorporated with downregulated expression of GDF10 compared to the upregulated expression of GDF10 counterparts (Fig. 6E). The analysis of PR positive status of relapse-free survival also determined that lower survival probability of breast cancer patients is directed according to a lower expression of GDF10 (Fig. 6F). Then, we went through a similar kind of analysis on the overall survival rate of breast cancer patients correlated with the GDF10. Here, we also found the positive correlation of GDF10 expression with the lower survival rate of breast cancer patients based on ER-negative and basal-like 2 status (Fig. 6 G-H). However, the resulting *p*-value of overall survival was not found as statistically significant. All of the outcomes were depicted in a forest plot based on HR value (Fig. 6I). By listing all the data corresponding to this meta-analysis, a significant association of GDF10 in the clinical prognosis of breast cancer was presented in Table 2.

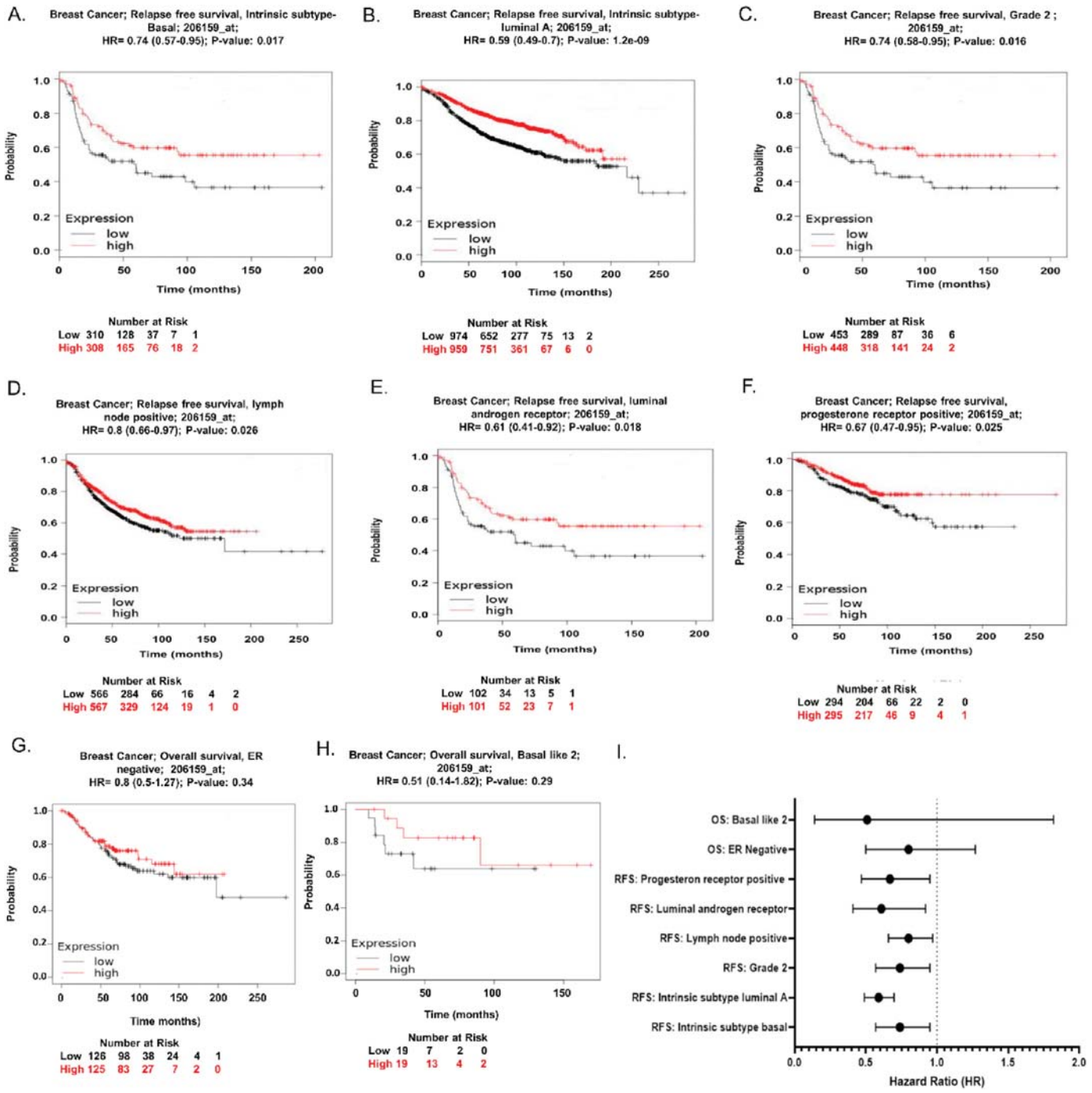

Fig. 6. The prognostic survival of breast cancer patients correlated with GDF10 expression. (A–H) Relapse free and overall survival probability was analyzed against multidisciplinary breast cancer types based on GDF10 expression. The survival curves demonstrated higher expression as a red line and lower expression as a black line. (I) The forest plot represented the outcomes of meta-analysis based on the hazard ratio (HR). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.7. Identification of the co-expressed genes of GDF10 associated with breast cancer development

To identify the co-expressed genes of GDF10 associated with breast cancer development, we focused on determining the genes which are positively correlated with the expression profile of GDF10. We found a list of 19 genes that are positively co-expressed with GDF10 (Supplementary Fig. 2A). Among all these genes, ABCA8 was most significantly co-expressed with the expression of GDF10 (R = 0.875). The bcGenExMiner webserver was used for graphical representation of Pearson's correlation (r = 0.45), in which the X-axis demonstrates GDF10 expression and the Y-axis reflects ABCA8 expression (Supplementary Fig. 2B). After that, we also verify the Pearson's correlation ratio (r = 0.8230) and Spearman's correlation ratio (r = 0.8432) using UCSC Xena which also revealed a significant relationship between GDF10 and ABCA8 expression (Supplementary Figs. 2C–D). Overall, the findings suggested that ABCA8 is one of the most significant co-expressed genes of GDF10 which may contribute to breast cancer development by any possible signaling pathway. The datasets exhibiting the co-expression relationship of ABCA8 with GDF10 are represented in Supplementary

Table 2
The correlation of GDF10 expression with the survival of breast cancer patients.

Clinicopathological characteristics	n	Hazard ratio	*p*-Value
Relapse Free Survival (RFS)			
All	3955	0.64 (0.57–0.71)	8.0e-16
ER Status			
ER Positive	2565	0.64 (0.44–0.93)	0.02
ER Negative	1214	0.87 (0.3–2.49)	0.8
HER2 Status			
HER2 negative	1456	0.77 (0.59–1.00)	0.052
HER2 positive	414	1.01 (0.65–1.57)	0.96
PR Status			
PR Positive	954	0.67 (0.47–0.95)	0.025
PR Negative	1028	0.98 (0.74–1.32)	0.91
Intrinsic Subtype			
Basal Type	879	0.74 (0.58–0.95)	0.016
Luminal A	2504	0.59 (0.49 − 0.7)	1.2e−09
Luminal B	1425	0.77 (0.63 − 0.93)	0.0072
HER2+	335	0.85 (0.58 − 1.24)	0.4
Stage 1	69	2.28 (0.83–6.3)	0.0102
Stage 2	145	1.89 (1.04–3.4)	0.032
Stage 3	319	1.92 (1.41–2.6)	3.0e-5
Stage 4	152	1.3 (0.88–1.93)	0.18
Lymph Node Status			
Lymph Node positive	1459	0.8 (0.66 − 0.97)	0.026
Lymph Node Negative	2259	0.8 (0.67 − 0.94)	0.0084
Grade			
1	378	0.76 (0.41 − 1.41)	0.38
2	1077	0.74 (0.58 − 0.95)	0.16
3	1090	1.14 (0.82 − 1.61)	0.44
TP53 Status			
Mutated	232	1.15 (0.37 − 3.61)	0.81
Wild Type	363	0.98 (0.64 − 1.49)	0.92
Pietenpol subtype			
luminal androgen receptor	276	0.61 (0.41–0.92)	0.018
Overall Survival (OS)			
ER negative	1214	0.8 (0.5–1.27)	0.34
Basal like 2	97	0.51 (0.14–1.82)	0.29

Table 4.

3.8. Determination of signaling pathways in breast cancer related to GDF10 and other co-expressed genes

Significant breast cancer-promoting pathways contributed by the GDF10 and related co-expressed genes were determined. The KEGG pathways explored predominant signaling pathways of breast cancer progression such as the pathways of cancer and cell cycle (Fig. 7A). Additionally, the top most reactome pathways signified GDF10 and other positively co-altered genes for incorporating with multidisciplinary pathways associated with cancer development such as cell cycle, extracellular matrix organization, mitotic cell cycle, developmental biology, hemostasis, mitotic metaphase and anaphase, M phase, mitotic anaphase, immune system and cell cycle checkpoints (Fig. 7B). Based on topmost panther pathways, we found that GDF10 and co-expressed genes were mostly related to Cadherin and Wnt signaling pathways (Fig. 7C).

Last of all, we examined the biological process of breast cancer prognosis by using the list of co-expressed genes of GDF10. In this case, we found the listed genes were most significantly associated with cellular response to cytokine stimulus in case of biological process (Fig. 7D), focal adhesion in terms of cellular component (Fig. 7E), and kinase binding in case of molecular function (Fig. 7F).

4. Discussion

Breast cancer has become the most threatening type of cancer for women, reflecting an estimation of 25.1% of total cancer patients. Surgery and other postoperative oncological steps such as radio therapy, endocrine therapy, and chemotherapy are the most common treatments for early-stage breast carcinoma. However, these adjuvant therapies may have some disadvantages and the majority of breast cancer patients face various clinicopathological problems such as pain, paranesthesia, and strange sensation [34,35]. Moreover, adjuvant endocrine therapy is linked to the reduction of bone minerals and may also lead to the skeletal morbidity. However, molecular targeted therapy has improved cure probability with less difficulty due to the working through selective inhibition of the targeted cancer cells [36,37].

Breast cancer is found mainly in two forms: (1) lobular and (2) ductal breast carcinomas. Clinically breast cancer is divided into three subtypes: (1) ERBB-2 amplified breast cancer, (2) tumor expressing progesterone receptors (PRs), or estrogen receptors (ERs), and (3) triple-negative breast cancer (TNBC) [38,39]. TNBC is the most unique, as it lacks PRs, ERs, and HER2 expression. By promoting the upregulation of the functional inhibitory approaches of Smad7, GDF10 suppresses the progression of TNBC [40].

Here, we have done an in-depth multi-omics analysis of the GDF10 expression pattern in breast cancer using variant numbers of publicly available bioinformatics tools. Interestingly in all cases, we found a downregulated expression of GDF10 in breast cancer. Thereafter, we have addressed the analysis of GDF10 expression for multiple types of breast cancer. Then we did a comparative study on immunohistochemistry in between breast cancer and normal tissues. In this investigation, we noted vast differences in staining, intensity, and quality of tumor cells as compared to normal glandular cells. Then, we evaluated GDF10 expression and promoter methylation levels based on individual clinicopathological characteristics. The outcome data of this analysis also suggested the downregulation of GDF10 mRNA expression and the upregulated promoter methylation level for each of the selected parameters in breast cancer. To explore the reasons behind these changes in gene expression, we addressed the functional characterization of GDF10 protein. From this study, we identified three missense types of mutation, and different other copy number alterations in 478 amino acid long human GDF10 protein, where the frequency of genetic changes ranges within 0.62–2.95%. These genetic changes may be significant markers of downregulated GDF10 expression in breast cancer. Following this, we studied the relationship between the survival probability of breast cancer patients correlated with GDF10 expression. The graphical outcomes of this study suggested a positive correlation between GDF10 expression and the survival probability of breast cancer patients.

To explore the progressive pathways of breast cancer correlated with GDF10, we analyzed the topmost co-expressed genes for GDF10. We identified 19 significantly co-expressed genes and also pointed out ABCA8 as the most significantly correlated gene. ABCA8 is a member of the ABC (ATP-binding cassette) superfamily. ABC has an association with membrane proteins, transportation, regulation, and cellular signaling [41,42]. The ABC family including ABCA8 is involved in cholesterol and lipid homeostasis, tumor and cancer progression. Lipid and cholesterols are essential to the cell membrane component required for maintaining the tumor microenvironment and cellular proliferation. They also contribute directly as the growth factor and indirectly as the signaling molecule to activate cancer pathways contributing to tumorigenesis [43,44]. However, the specific oncogenic role of ABCA8 interconnecting with the GDF10 is poorly defined.

Afterward, we determined the significant pathways induced by GDF10 and its topmost co-expressed genes. In most of the cases, we found that GDF10 and its co-expressed genes are related to the signaling pathways associated with cancer development. Based on the KEGG pathway, we found the association between GDF10 and its positively co-expressed genes with the pathways directly involved in cancer. This pathway analysis strongly suggested the direct interconnection of GDF10 with cancer development. From the Reactome pathways, we experienced the relation of GDF10 and all other co-expressed genes with the cell cycle. The Cell cycle is a fundamental biological pathway with dysfunction that is interlinked with the cancer development through the promotion of malignant growth. The dysfunction of the cell cycle may be mediated by the mutagenic, downregulated expression of the GDF10

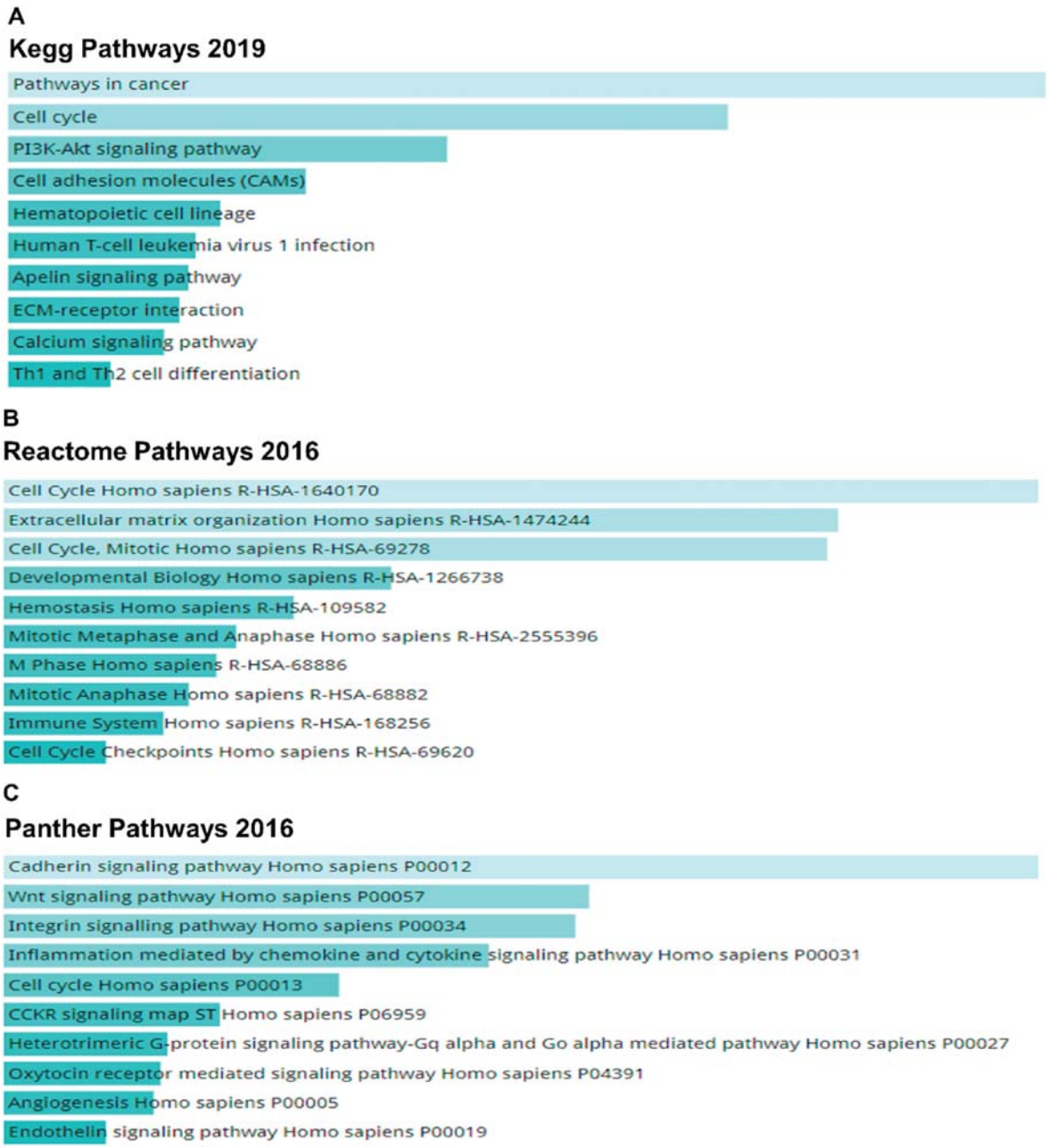

Fig. 7. Signaling pathway of GDF10 and co-expressed genes. The bar length represents the significance of that term and brighter color represents more significance. (A). KEGG 2019 pathway, (B). Reactome Pathway, (C). Panther 2016 pathway. (D). GO Biological Process 2018 (E). GO Cellular Component 2018 (F). GO Molecular Function 2018. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

tumor suppressive gene and their co-expressed genes. The PANTHER pathway indicated the most probable interconnection of GDF10 is with the cadherin signaling pathway and Wnt signaling pathway. A recent study suggested that the β-Cadherin and Wnt signaling pathways are related to cancer development. More specifically, these pathways contribute to breast cancer progression through the TGF-β signaling pathway. The vascular cadherin protein promotes the TGF-β signaling pathway that mediates breast cancer progression by utilizing endothelial mesenchymal transmission (EMT). The TGF-β signaling pathway also enhances the activation of the oncogenic Wnt signaling pathway by inhibiting Dickkopf related protein 1 (DKK1) [45,46]. The TGF-β signaling pathway is promoted by Smad2/Smad4 complexes. In this case, Smad7 acts as the suppressor of the TGF-β signaling pathway by inhibiting the expression of Smad2/Smad4 complexes. GDF10 works to stimulate the expression of Smad7 and helps to suppress the TGF-β signaling pathway. However, in the carcinogenic condition, the down-regulated GDF10 expression cannot favor the expression of Smad7. Therefore, the TGF-β signaling pathway remains functional to interconnect with the cadherin signaling pathway and the Wnt signaling pathway [45,47]. The promotion of the Wnt signaling pathway and cadherin signaling pathway through down-expressed GDF10 is predicted as a potential reason for the progression of breast cancer (Fig. 8). Thereafter, we have addressed GO analysis for investigating the most significant functions of GDF10 and other co-expressed genes at the biological, cellular, and molecular levels. In the case of the biological process, we found the maximum corporation of GDF10 is with cellular response to cytokine stimulus. Breast cells are capable of cytokine production and these cytokines can act as tumor inhibiting or

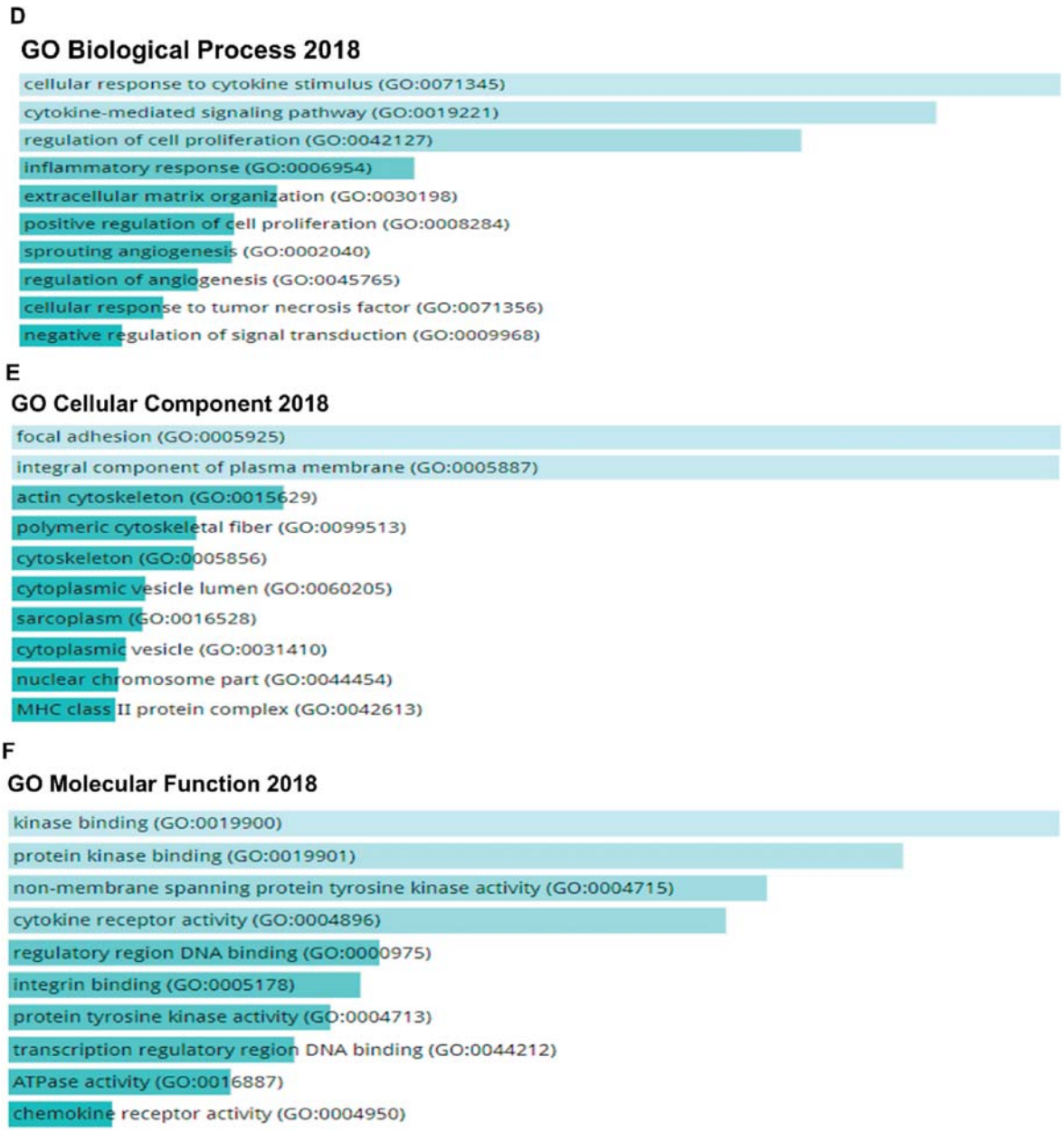

Fig. 7. (*continued*).

growth-promoting factors. As GDF10 is associated with cellular response to cytokine stimulus, and cytokines are involved in tumor growth regulation, so GDF10 expression is assumed to be indirectly associated with tumor growth processing [48]. Moreover, based on the cellular component, we determined that the topmost relation of GDF10 is along with focal adhesion, which is a prominent determinant of breast cancer commencement, metastasis, and progression. According to this report and GO analysis of the cellular component, an interlink is suggested between GDF10 with breast cancer as it is related to focal adhesion [49]. The GO molecular function study revealed that GDF10 and all its relevant co-altered genes in breast carcinoma are mostly associated with kinase binding. Protein tyrosine kinases are reported as the tightly regulated catalyst in breast cancer due to oncogenic mutation. Therefore, GDF10 may be incorporated with breast cancer as it interacts with kinase binding. Overall, all of these analyses suggested that GDF10 can be utilized as a possible biomarker for breast cancer. However, the discovery of more prevalent molecular knowledge about the functional role and oncological significance of GDF10 in breast cancer is desirable.

5. Conclusion

In this multidisciplinary research study, we utilized several bioinformatics tools to justify the efficiency of GDF10 to be a novel therapeutic biomarker for breast cancer. We performed a systematic multi-omics analysis to examine the expression level, methylation status, immunohistochemistry, functional attitude through mutation and copy number alteration analysis, prognostic significance, and their impact on variant signaling pathways associated with breast cancer development. Present outcomes of the study explored GDF10 as a potential tumor suppressor whose expression is downregulated significantly in breast cancer developing tissues. Elevated methylation levels and other changes in genetic level such as mutations or copy number alterations may be responsible for promoting this decreased expression of GDF10. In words, the ultimate outcomes of this research approach determined the efficiency of GDF10 as a novel therapeutic biomarker for breast cancer. Hence, more relevant wet laboratory-based studies are expected to emphasize the significance of GDF10 for early diagnosis and

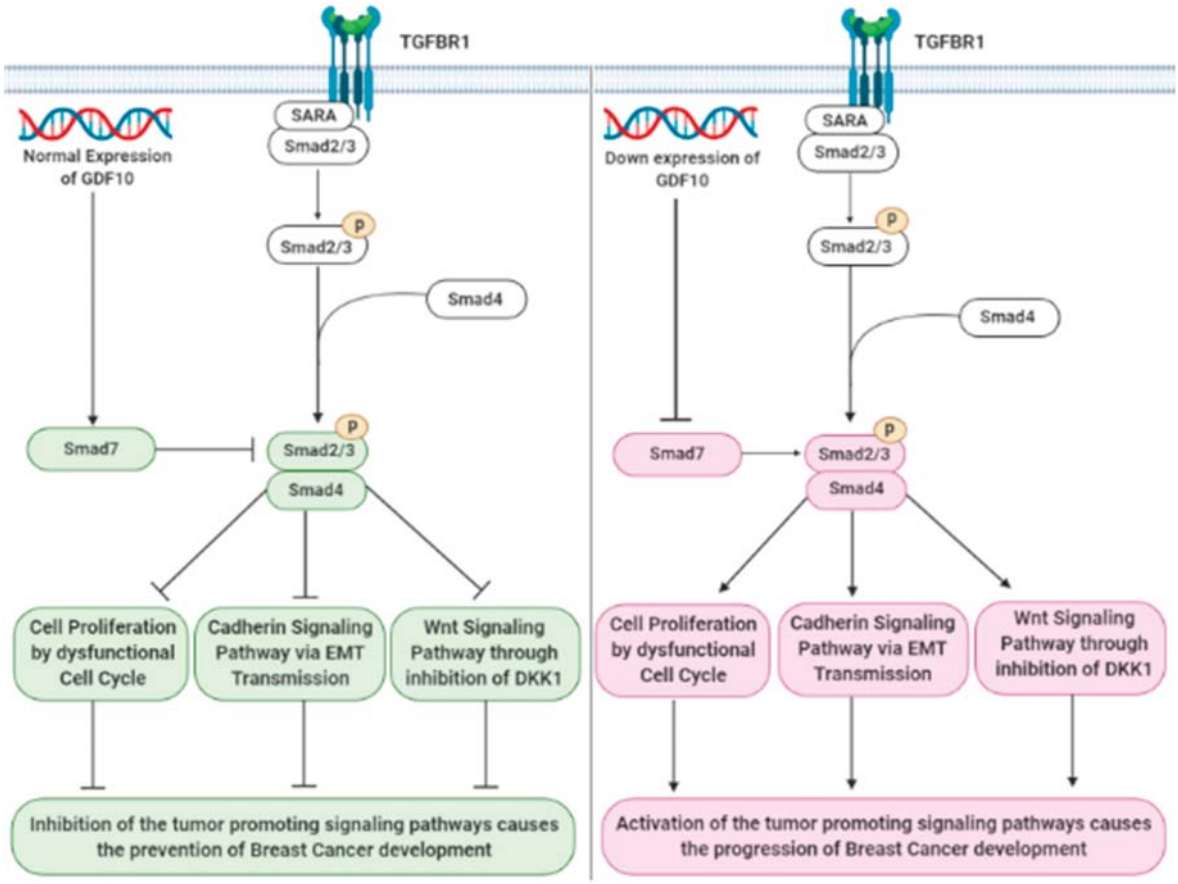

Fig. 8. A schematic illustration representing the impact of GDF10 expression in breast cancer development. In normal conditions, GDF10 stimulates Smad7 to inhibit the formation of Smad2/Smad4 complexes. But in carcinogenic conditions, the down expressed GDF10 cannot trigger the action of Smad7. Therefore, Smad2/Smad4 complexes become involved in breast cancer-causing signaling pathways, which ultimately promotes the progression of breast cancer development.

improved treatment strategy of breast cancer.

Authors contributions

AS and FA designed the project; all authors contributed to generating and analyzing the data. FR wrote the introduction and methodology result of the manuscript; TBM wrote the result and discussion part of the manuscript; RA, AS, and FA reviewed the manuscript. All authors read and approved the final manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.imu.2020.100463.

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