Original Article

Function and prognosis analysis of nucleolus protein DCAF13 in breast cancer

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Background: Breast cancer is one of the main causes of death among women. RNA binding proteins (RBPs) play a crucial role in the progression of breast cancer, with increasingly detailed understanding of RBP functional molecular mechanisms in breast cancer, the functional research of RBPs may help elucidate the potential mechanisms of tumor occurrence, development, invasion, metastasis and prognosis. DDB1- and CUL4-associated factor 13 (DCAF13) is an RBPs has been identified as a substrate receptor for the CUL4-DDB1 E3 ligase complex. Its expression is related to the prognosis of certain cancer. We tried to explore both co-expressed network and biological functions of DCAF13 in breast cancer.

Methods: The Cancer Genome Atlas (TCGA) database was used to analyze the different expression of DCAF13 messenger RNA (mRNA) between normal breast tissue and breast carcinoma tissue, and the clinical data about 960 samples were downloaded from the cBio Cancer Genomics Portal (cBioPortal). The expression level of DCAF13, co-expression network, and survival were analyzed. Those with a fold change ≥1 and FDR <0.05 were considered to have statistical significance. Unsupervised clustering of differentially expressed RBPs was performed based on log2-transformed FPKM values using the "pheatmap" package in R. Genes with a Spearman score >0.55 were regarded as moderately co-expressed genes. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database was used to construct a co-expression network. Meanwhile, the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases were used to identify the biological process cluster and pathway cluster, respectively.

Results: Compared with normal breast tissue, DCAF13 mRNA expression was significantly increased in breast cancer tissue (P<0.01). The Database for Annotation, Visualization and Integrated Discovery (DAVID) was used to identify the functions of the co-expressed network. These genes were mainly enriched in mitosis, nuclear division, metabolic process, recombination, replication and repair of DNA, double-strand break repair, posttranscriptional regulation of gene expression, regulation of cell cycle, division and proliferation, regulation of protein stability and also participation in in regulation of poly(A) RNA binding, mRNA binding, tRNA binding, adenosine triphosphate (ATP) binding. KEGG pathway analysis revealed that the genes were mainly enriched in cell cycle, oocyte meiosis and oxidative phosphorylation. According to survival analysis, upregulation of DCAF13 mRNA was significant for overall survival (OS) (P=0.0163).

Conclusions: DCAF13 is up-regulated in breast cancer, the OS of patients with DCAF13 up-regulation was obviously reduced. DCAF13 was used as a diagnostic marker and therapeutic target for breast cancer. By building a co-expression network of DCAF13 and conducting bioinformatics analysis, it is possible to find the biomarker to evaluate patient prognosis. This finding provides a new target in mechanism and cell research of breast cancer.

Keywords: Breast cancer; DDB1- and CUL4-associated factor 13 (DCAF13); co-expression network; survival; prognosis

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Introduction

Worldwide, there will be an estimated 2.26 million newly diagnosed female breast cancer cases in 2022, accounting for almost 1 in 4 cancer cases among women. Breast cancer is the leading cause of cancer death in more than 100 countries (1). With the great progress of medical technology, the diagnosis incidence of breast cancer has been increasing year by year, and the age of onset is becoming markedly younger.

RNA binding proteins (RBPs) were known that post-transcription contributes to tumor initiation and progression, the role of RBPs in cancer remain relatively unexplored. DDB1- and CUL4-associated factor 13 (DCAF13) is an RBP widely amplified in a variety of cancers. Some studies have supported that DCAF13 being an oncogene. DCAF13 is an RBPs has been identified as a substrate receptor for the CUL4-DDB1 E3 ligase complex. Its expression is related to the prognosis of certain cancers, DCAF13 is overexpressed in hepatocellular carcinoma and lung adenocarcinoma, knockdown of DCAF13 inhibited the migration of human lung adenocarcinoma cells.

In order to reduce the mortality and recurrence of breast cancer patients, and even improve their quality of life, it is crucial to enhance early diagnosis and prognosis monitoring. In recent years, the study of biological markers and functional genes has remained a hot spot in breast cancer research (2). Currently, the study of molecular biomarkers for diagnosis, treatment, and prognosis is increasing, and it is important to identify markers to evaluate tumor development. This study used The Cancer Genome Atlas (TCGA), cBioPortal, and Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; www.string.org) databases to explore the expression relationship between DCAF13 and breast cancer and determine the biological function of its co-expression network. We present this article in accordance with the REMARK reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-1923/rc).

Highlight box

Key findings

- DCAF13 mRNA is significantly over-expressed in breast cancer than in normal tissue.
- The DAVID database has been used to identify the functions of the co-expressed network. These genes were mainly enriched in mitosis, nuclear division, metabolic process, recombination, replication and repair of DNA, double-strand break repair, posttranscriptional regulation of gene expression, regulation of cell cycle, regulation of protein stability, division and proliferation.
- Patients with up-regulation of DCAF13 might have lower survival and poor prognosis.

What is known and what is new?

- DCAF13 is overexpressed in many cancers, downregulation DCAF13 in breast cancer cell lines were markedly reduced breast cancer cell proliferation, clone formation, and migration.
- Clinical data obtained from TCGA was used to analysis the function of DCAF13 in breast cancer patients. DCAF13 mRNA was significantly over-expressed in breast cancer than in normal tissue. Patients with up-regulation of DCAF13 might have lower survival and poor prognosis.

What is the implication, and what should change now?

- DCAF13 plays an important role in breast cancer proliferation, invasion and migration in the clinical data. DCAF13 was used as a diagnostic marker and therapeutic target for breast cancer treatment.

Methods

Data sources analysis

In our previous study, 1,092 breast tumor samples and 113 normal controls were used to study role of RBPs in the occurrence and development of breast cancer. We identified 90 upregulated and 115 downregulated RBPs in breast cancer. Survival analysis found that overexpression of DCAF13, EZR, and MRPL13 showed worse survival, but the mechanism of DCAF13 in the occurrence and development of breast cancer is unknown (3). So, TCGA visualization online database (cBioPortal; http://www.cbioportal.org/) was used for further analysis the function of DCAF13 in the

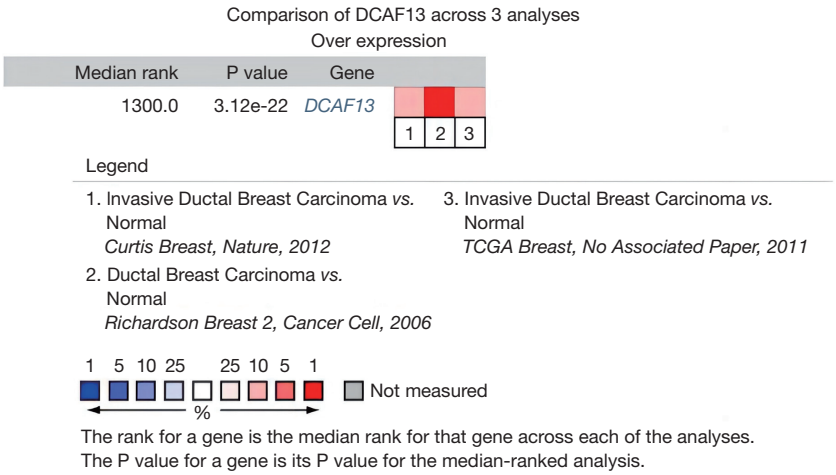

Figure 1 Three studies about the differential expression of DCAF13 mRNA between healthy tissue and breast cancer. mRNA, messenger RNA; TCGA, The Cancer Genome Atlas.

breast cancer. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Constructed the co-expression network

The STRING database was used to obtain co-expression groups, and a protein-protein interaction (PPI) network was constructed by Cytoscape (https://cytoscape.org/).

The function and pathway analysis of PPI

To better understand biological function of the PPI, we used the Database for Annotation, Visualization and Integrated Discovery (DAVID; http://david.ncifcrf.gov/) to conduct pathway and function analysis of co-expression genes; the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases were used to identify biological process and pathway enrichment, respectively.

Statistical analysis

In the pathway and functional enrichment analysis, P<0.05 was considered statistically significant. Survival analysis was conducted using the Kaplan-Meier online tool (http://www.kmplot.com/), and P<0.05 was considered statistically significant.

Results

The expression of DCAF13 between normal tissue and breast cancer

We identified that three studies have involved DCAF13: Richardson breast (4), TCGA breast (4-6), and Curtis breast (7-11). A review of these three studies found that DCAF13 mRNA was obviously over-expressed in breast cancer, with a median rank of 1,300.0, P=3.12e–22 (*Figure 1*). Then, a box plot constructed to compare the 2,197 samples revealed that compared with normal tissue, DCAF13 was significantly up-regulated in breast cancer (P<0.01) (*Figure 2A-2C*).

In the cBioPortal database, about 45% of 960 samples had alteration of DCAF13 and were mainly up-regulated (*Figure 3*), which was consistent with the increase of the copy number of DCAF13 (*Figure 4*).

Screened and constructed co-expression network

In this study, genes with a Spearman correlation coefficient >0.55 were regarded as the moderate level. Next, to get their co-expressed interaction groups were identified though the STRING database; finally, 100 nodes were obtained in the PPI and the P value of PPI concentration was 4.3E–08 (*Figure 5*). Then, the co-expression network was visualized using Cytoscape 3.6.

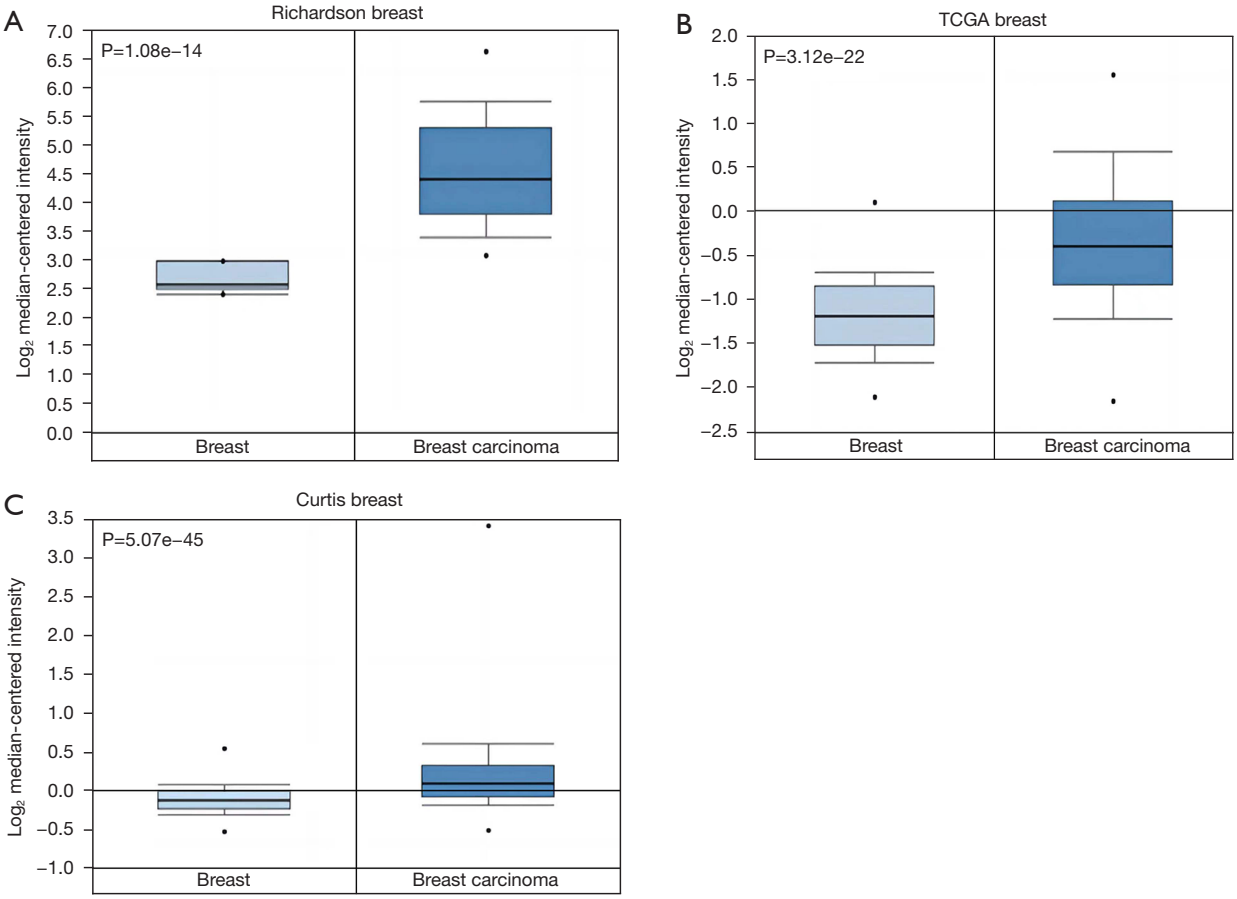

Figure 2 The expressed level of DCAF13 mRNA between normal tissue and breast cancer. (A) Richardson breast, (B) TCGA breast, (C) Curtis breast. TCGA, The Cancer Genome Atlas; mRNA, RNA, messenger RNA.

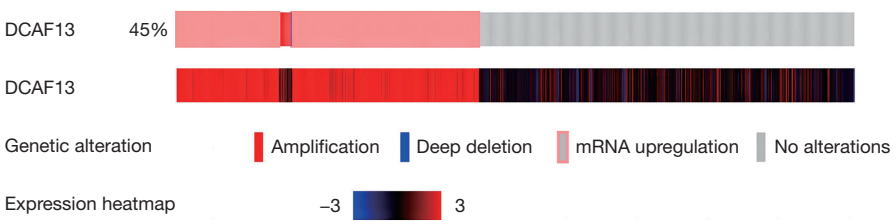

Figure 3 The expression of DCAF13 in breast cancer. mRNA, messenger RNA.

The biological function and pathway analysis of the co-expressed network

The DAVID database was used to identify the functions of the co-expressed network. For the BP, these genes were mainly enriched in mitosis, nuclear division, metabolic process, recombination, replication and repair of DNA, double-strand break repair, posttranscriptional regulation of gene expression, regulation of cell cycle, division and proliferation, regulation of protein stability, and so on (*Table 1*). The cellular components (CCs) were significantly located in nuclear and organelle lumen, nucleoplasm, nucleolus, eukaryotic translation initiation factor 3 complex, chromosome, mitochondrion, cytosol, and so on (*Table 2*). For molecular functions (MFs), the results

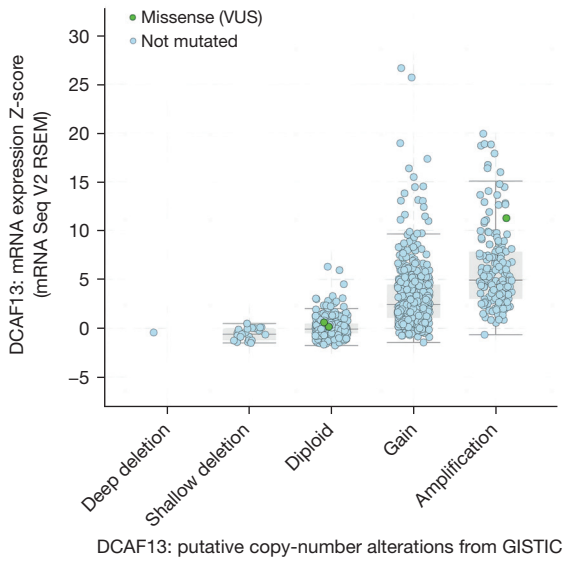

Figure 4 The relationship between copy number of DCAF13 and expression level of mRNA. mRNA, messenger RNA; RSEM, RNA-Seq by Expectation-Maximization; GISTIC, Genomic Identification of Significant Targets in Cancer; VUS, Missense Variants of Uncertain Significance.

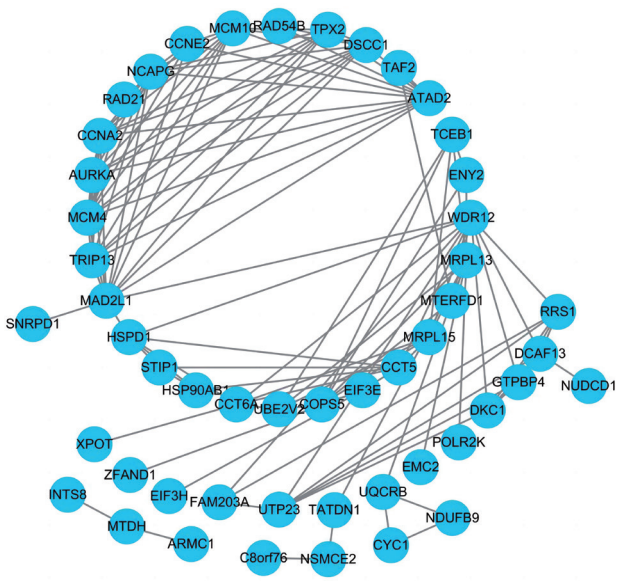

Figure 5 The co-expression network of DCAF13 (PPI). PPI, protein-protein interaction.

showed that these genes mainly participated in nucleotide binding, nucleoside binding, poly-purine tract binding, and adenosine triphosphate (ATP) binding (*Table 3*).

Table 1 The biological progress of co-expressed genes

ID	Term	P value
GO:0006259	DNA metabolic process	1.31E−05
GO:0007067	Mitosis	2.11E−05
GO:0000280	Nuclear division	2.11E−05
GO:0006396	RNA processing	1.37E−04
GO:0007049	Cell cycle	2.01E−04
GO:0006310	DNA recombination	2.35E−03
GO:0006260	DNA replication	3.39E−03
GO:0006302	Double-strand break repair	4.37E−03
GO:0010608	Posttranscriptional regulation of gene expression	5.28E−03
GO:0051726	Regulation of cell cycle	8.27E−03
GO:0000075	Cell cycle checkpoint	1.26E−02
GO:0016071	mRNA metabolic process	1.38E−02
GO:0006281	DNA repair	1.76E−02
GO:0051301	Cell division	2.03E−02
GO:0031647	Regulation of protein stability	2.10E−02
GO:0008283	Cell proliferation	2.83E−02

mRNA, messenger RNA; GO, Gene Ontology.

Table 2 The cellular components of co-expressed genes

ID	Term	P value
GO:0031981	Nuclear lumen	2.61E−09
GO:0043233	Organelle lumen	1.50E−08
GO:0005654	Nucleoplasm	1.25E−06
GO:0005730	Nucleolus	7.15E−04
GO:0005852	Eukaryotic translation initiation factor 3 complex	2.56E−03
GO:0005694	Chromosome	8.59E−03
GO:0031966	Mitochondrial membrane	1.48E−02
GO:0005739	Mitochondrion	2.02E−02
GO:0019866	Organelle inner membrane	2.57E−02
GO:0005829	Cytosol	3.42E−02

GO, Gene Ontology.

KEGG pathway analysis revealed that the genes were mainly enriched in cell cycle, oocyte meiosis and oxidative phosphorylation (*Table 4*).

Table 3 Molecular functions of co-expressed genes

ID	Term	P value
GO:0000166	Nucleotide binding	6.93E−03
GO:0001882	Nucleoside binding	1.79E−02
GO:0003743	Translation initiation factor activity	3.43E−02
GO:0008134	Transcription factor binding	3.51E−02
GO:0008143	Poly(A) RNA binding	3.76E−02
GO:0003729	mRNA binding	3.96E−02
GO:0070717	Poly-purine tract binding	4.22E−02
GO:0005524	ATP binding	4.46E−02

GO, Gene Ontology; ATP, adenosine triphosphate.

Table 4 KEGG pathway analysis of co-expressed genes

ID	Term	P value
hsa04110	Cell cycle	7.16E−05
hsa04114	Oocyte meiosis	2.62E−02
hsa00190	Oxidative phosphorylation	4.01E−02

KEGG, Kyoto Encyclopedia of Genes and Genomes.

The relationship between DCAF13 mRNA and prognosis in breast cancer

Over-expressed DCAF13 mRNA was closely related with overall survival (OS; P=0.0163). Compared with other patients, the OS of patients with DCAF13 up-regulation was obviously reduced, indicating a poor prognosis (*Figure 6*).

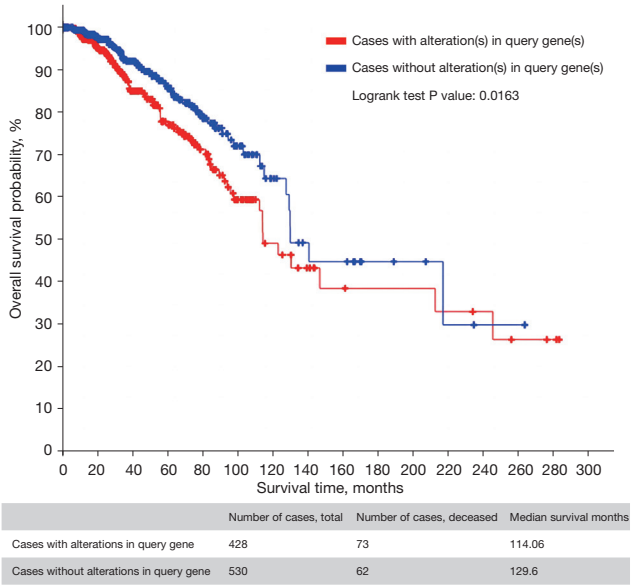

Figure 6 The relationship between DCAF13 expression and patients' prognosis in breast cancer.

Discussion

Breast cancer is the most commonly diagnosed cancer and the main cause of cancer death among women. In China, about 280,000 new breast cancer patients are diagnosed yearly. The incidence age of Chinese women is about 10 years earlier than that of European women, and most patients are diagnosed before menopause; breast cancer has become the prevalent tumor affecting females in China (11,12). Many studies have demonstrated that breast cancer is caused by multiple genes, multiple pathways, and multiple influencing factors. In recent years, the phenomenon that the incidence of breast cancer is gradually increasing has attracted great attention, and there is an urgent need to clarify the molecular mechanism and develop new examination and treatment strategies.

DCAF13 is on chromosome 22,q13.1 in the human genome and also is one of RBPs. Many studies have shown that DCAF13 plays an important role in the maintenance and growth of oocytes and is related with infertility in women, the deletion of which can result in disordered ribosome assembly (12,13). DCAF13 is amplified in a variety of cancers. Some studies have supported that DCAF13 being an oncogene. Research has reported that DCAF13 is overexpressed in hepatocellular carcinoma and can be an independent predictor of poor survival in hepatocellular carcinoma. It is also significantly upregulated in human lung adenocarcinoma and knockdown of DCAF13 inhibited the migration of human lung adenocarcinoma cells (5). In addition, research has demonstrated that an E3 ligase consisting of DNA damage binding protein 1 (DDB1), DCAF13, and CUL4B, which can specifically recognize PTEN as a substrate for ubiquitination in human osteosarcoma cells and disrupt this E3 ligase might result in PTEN accumulation (14). It is well-known that abnormality and inactivation of tumor suppressor genes are crucial factors of tumorigenesis. Many studies have suggested that PTEN might regulate many signal pathways, including the FAK pathway, PIP3 pathway, Wnt/β-catenin, and MAPK pathways (11,13-15). Aberrant PTEN can result in various cancers, such as breast cancer, endometrial adenocarcinoma,

and hepatocellular carcinoma. However, the development of breast cancer is correlated with abnormal signaling pathways, for example, Wnt/β-catenin participates in occurrence, invasion, and even drug resistance of breast cancer (16,17). Therefore, the role of DCAF13 in cancer should not be ignored and it is worthwhile to explore its function and mechanism in breast cancer.

Most importantly, this study found that DCAF13 mRNA was significantly over-expressed in breast cancer than in normal tissue through cBioPortal database and was also closely related with OS, indicating that patients with up-regulation of DCAF13 might have lower survival and poor prognosis. Our research not only reveals that DCAF13 promotes the breast cancer carcinogenesis but also helps to further study the molecular biological mechanism. For the biological function, GO analysis showed that it was mainly enriched in regulation of cell cycle and mitotic cycle, metabolic process, recombination, replication and repair of DNA, posttranscriptional regulation of gene expression, translation initiation factor activity, transcription factor binding, poly(A) RNA binding, mRNA binding, oxidative phosphorylation, both biological function and signaling pathway were closely correlated with abnormal cell proliferation and epigenesis, which suggests that DCAF13 may the important indicator of poor prognosis in breast cancer. Exploration of the molecular and cellular regulation mechanisms of the occurrence, development, and metastasis in breast cancer may provide potential biomarkers for clinical diagnosis, prognosis assessment, and targeted therapy of breast cancer (18,19). However, the mechanism of DCAF13 promoting proliferation, invasion and metastasis of breast cancer is unknown. DCAF13 promote the canceration of breast cancer need to be further confirmed *in vitro* and *in vivo*. So, further experiments are required to verify the function of DCAF13.

Conclusions

DCAF13 mRNA was significantly over-expressed in breast cancer than in normal tissue. By building a co-expression network of DCAF13 and conducting bioinformatics analysis, these genes were mainly enriched in regulation of cell cycle and mitotic cycle, metabolic process, recombination, replication and repair of DNA and so on. Patients with up-regulation of DCAF13 might have lower survival and poor prognosis. It is possible to find the biomarker to evaluate patient prognosis. This finding provides a new target in mechanism and cell research of breast cancer.

Acknowledgments

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-1923/rc

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-1923/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-1923/coif). L.R. received financial support from Novartis, Gilead and Seagen for advisory boards. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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