DEAD-Box Helicase 27 Enhances Stem Cell-Like Properties With Poor Prognosis in Breast Cancer

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Abstract

Background

Although the rapid development of diagnosis and treatment has improved prognosis in early breast cancer, challenges from different therapy response remain due to breast cancer heterogeneity. DEAD-box helicase 27 (DDX27) had been proved to influence ribosome biogenesis and identified as a promoter in gastric and colorectal cancer associated with stem cell-like properties, while the impact of DDX27 on breast cancer prognosis and biological functions is unclear. We aimed to explore the influence of DDX27 on stem cell-like properties and prognosis in breast cancer.

Methods

The expression of DDX27 was evaluated in 24 pairs of fresh breast cancer and normal tissue by western blot. We conducted Immunohistochemical (IHC) staining in paraffin sections of 165 breast cancer patients to analyze the expression of DDX27 and its correlation to stemness biomarker. The Cancer Genome Atlas-Brest Cancer (TCGA-BRCA) and Kaplan-Meier survival analysis were used to investigate the expression of DDX27 in breast cancer and its implication on prognosis. Western blot, CCK-8 assay, Transwell assay and wound-healing assay were used to clarify the regulation of DDX27 on stem cell-like properties in breast cancer cells. Gene Set Enrichment Analysis (GSEA) was performed to analyze the potential molecular mechanisms of DDX27 in breast cancer.

Results

DDX27 was significantly high expressed in breast cancer compared with normal tissue. High expression of DDX27 was related to larger tumor size ($p = 0.0005$), positive lymph nodes ($p = 0.0008$), higher histological grade ($p = 0.0040$), higher ki-67 ($p = 0.0063$) and later TNM stage ($p \square 0.0001$). Patients with high DDX27 expression turned out worse prognosis on overall survival (OS, $p = 0.0087$) and disease-free survival (DFS, $p = 0.0235$). Overexpression of DDX27 could enhance the expression of biomarkers related to stemness and promote stem cell-like activities such as proliferation and migration in breast cancer cells.

Conclusion

DDX27 can enhance stem cell-like properties and cause poor prognosis in breast cancer, also may be expected to become a potential biomarker for breast cancer therapy.

Background

Breast cancer has already surpassed lung cancer and developed into the major malignant tumor in women all around the world with 2.3 million new cases in 2020 based on GLOBOCAN 2020[1]. Although the rapid development of diagnosis and treatment has improved prognosis in early breast cancer, challenges from different therapy response remain due to breast cancer heterogeneity.

DEAD-box helicase 27 (DDX27) pertains to the DEAD-box RNA helicases family, which is a classical ATP-dependent helicases family containing conserved D-E-A-D (Asp-Glu-Ala-Asp) sequences. This family has been confirmed to participate in various processes containing RNA transportation, RNA degradation, glucose metabolism, lipid metabolism, ribosome biosynthesis, tumorigenesis, cancer development and so on[2–8]. DDX27 was proved to take part in the processes of ribosome biogenesis, which performed an important function in cell proliferating. DDX27 could regulate the 47S ribosome RNA formation and associated with PeBow-complex independently[9]. In the process of skeletal muscle myogenesis, DDX27 was reported to influence ribosome RNA maturation, ribosome biogenesis and specific transcription[10]. DDX27 was also related to the development of malignant tumor. DDX27 had been confirmed to promote the development and metastasis in hepatocellular carcinoma and gastrointestinal cancer with poor prognosis[11–13]. Until now, the status of DDX27 expression and its implication on breast cancer remains unclear.

Breast cancer stem cells refer to a fraction of cells who has strong self-renewal capacity and a huge potential of multiple differentiation in breast cancer which can promote the courses of tumorigenesis, development, metastasis and drug resistance[14–16]. Studies proved that members of DEAD-box RNA helicases family could affect the biological behavior of cancer stem cells in various cancers[17–19]. The only member of DEAD-box RNA helicases family which has exactly effects on breast cancer stem cells is DDX17, who can enhance stem cell-like activities by combining with SOX2 or mechanism of promoting stem-like properties under hypoxia[20, 21]. DDX27 was reported to promote stem cell-like characteristics and cause poor prognosis in gastric and colorectal cancer[11, 22], while the influence on stemness in breast cancer remains unclear. Hence, it is of great significance to explore the influence of DDX27 on tumorigenesis, progress and the association with stem cell-like properties in breast cancer, which might suggest a new idea for diagnosis and therapy.

In our study, DDX27 was highly expressed in both The Cancer Genome Atlas-Brest Cancer (TCGA-BRCA) database and breast cancer samples. High expression of DDX27 was firstly reported in breast cancer with the connection to clinicopathological factors and caused a shorter survival. The expression of DDX27 was positively related to stemness biomarkers and promoted the stem cell-like activities. Furthermore, Gene Set Enrichment Analysis (GSEA) suggested that DDX27 might have an effect on breast cancer by various ways. Hence, DDX27 can enhance the stem cell-like properties meanwhile leading to a poor prognosis in breast cancer, which means DDX27 may become a potentially significant prognosis biomarker and therapeutic target.

Methods

The Cancer Genome Atlas-Brest Cancer Database

All of the gene expression data were downloaded and organized from TCGA-BRCA database (https://cancergenome.nih.gov/), which contains 1109 breast cancer and 103 normal samples. Normalization of all data was performed by edgR package.

Kaplan-Meier survival analysis and Gene Set Enrichment Analysis

Kaplan-Meier Plotter (http://kmplot.com/analysis/) is an online database containing survival information and gene expression of various cancers and used to analyze the implication of DDX27 on breast cancer prognosis [23]. The potential mechanism of DDX27 expression in breast cancer was analyzed by GSEA 4.0.3 software.

Patients and tissue samples

Under the permission from the Ethics Review Committee in the First Affiliated Hospital of China Medical University (AF - SOP – 07–1.1–01), fresh cancer tissue with paired adjacent normal tissue (n = 24), and paraffin-embedded specimens (n = 165) were obtained. All included patients were pathologically diagnosed as infiltrative ductal carcinoma. None of the patients received breast-conserving surgery or neoadjuvant therapy. Twenty-four pairs of fresh specimens were collected within 30 minutes after surgery and 165 paraffin-embedded specimens for IHC were collected during Jan.2014 to Dec.2015. Clinical and pathological information obtained from the Hospital Information System included age, tumor size, lymph nodes status, estrogen receptor (ER) status, progesterone receptor (PR) status, human epidermal growth factor receptor 2 (HER2) status, histological grade, ki-67 index and TNM stage.

Western blot

Samples of tissue and cells were lysed by the compound of RIPA buffer and protease inhibitors (Sigma-Aldrich) for half an hour on ice after washed by phosphate-buffered saline (PBS) twice. Then the samples were centrifuged for 15 minutes in the condition of 14,000×g and 4°C. Protein from tissue and cells was quantified by BCA assay kit (Beyotime, Jiangsu, China). Protein with equal quantity was transferred onto the polyvinylidene fluoride (PVDF) membranes (Millipore, Bedford, MA) after electrophoresed by SDS-PAGE. The membrane was sealed with bovine serum albumin (BSA) and hatched with primary antibodies at 4°C overnight, then the secondary antibodies at room temperature for one hour. The primary antibodies for experiments contained anti-DDX27 (1:2000, Abcam, USA), anti-SOX2 (1:1000, Proteintech, China), anti-OCT4 (1:1000, Cell Signaling Technology, USA) and β-actin (1:2000, Proteintech, China). Membranes were tested with an enhanced chemiluminescence detection kit (BOSTER, USA).

Immunohistochemical staining

Each paraffin-embedded sample was cut into 4μm sections, dewaxed routinely, and dehydrated with gradient ethanol. All sections were retrieved antigen in citrate buffer with high pressure. The sections were restored to room temperature and blocked the activity of endogenous peroxidase by 3% hydrogen

peroxide. Then, all the slides were incubated with anti-DDX27 (1:2000, Novus Biologicals, USA) or anti-OCT4 (1:500, Cell Signaling Technology, USA) at 4°C overnight. The sections were incubated with the secondary antibody at room temperature for one hour after the slides were washed by PBS on the second day. Sections were stained by diaminobenzidine (DAB) and counterstained by hematoxylin. Finally, slides were observed and captured with microscope.

Evaluation of IHC

Sections with DAB staining were evaluated blinded by two pathologists separately. The expression of DDX27 and OCT4 was assessed on the basis of the intensity of staining and the ration of positive stained cells. The intensity was divided into deep (3), medium (2), light (1) and negative (0). The positive stained cells were scored as follow: 0 (0−5%), 1 (6% − 25%), 2 (26% − 50%), 3 (51% − 75%), 4 (76% − 100%). Final score of IHC staining was given by multiplication of percentage score and intensity. In this way, 165 patients were segmented into different groups in accordance with the expression of DDX27 and OCT4: high expression (final score ≥ 4) and low expression (final score ≤ 3).

Cell lines and culture

Breast cancer cell lines MCF-7 and T47D gained from the American Type Culture Collection (ATCC) were cultured by high-glucose (4.5 mg/ml) DMEM (HyClone, USA) with 10% serum (Tianjin Hao Yang Biological Manufacture CL, China). MCF-7 mammosphere (MCF-7 MS) and T47D mammosphere (T47D MS) were inducted and cultured by DMED-F12 (Gibco) with EGF 20 µg/L (Promega), b-FGF 10 µg/L (Promega) and 2% B27 (Gibco). All of the cells used in our research were cultured in the condition containing 5% CO_2 and 95% air at 37°C.

Cell transfection

MCF-7 and T47D cells were transferred with over-expression and negative control plasmid (Genechem, Shanghai, China) using Lipofectamine 3000 (Thermo, USA).

Wound-healing assay

DDX27 over-expression and negative control plasmid were transfected into cells until 70% confluency in six-well plates. Linear "scratches" were created in straight lines with sterile tips. Then, cells were added serum-free medium after washing with PBS three times, and photographed by microscope at 0 h, 24 h, 48 h and 72h. The area of wound-healing was analyzed by Image J.

Transwell assay

MCF-7 and T47D transfected with DDX27 over-expression and negative control plasmid were starved with serum-free medium for 4 hours. 100µL serum-free medium with 20,000 cells and 600µL medium with 10% serum were added into the upper and lower chamber separately. All the cells migrated to the lower chamber were fixed and stained by paraformaldehyde and crystal violet 48 hours later. Three random fields were counted by Image J software for each chamber.

Cell Counting Kit-8 assay

Cell Counting Kit-8 (CCK-8) assay (Dojindo, Kumomoto, Japan) was performed to test the proliferation capability of cells. 2,000 cells transfected with DDX27 over-expression and negative control plasmid were seeded in 96-well plates each well. After 24h, 48h and 72h of transfection, we added 10μL CCK-8 solution in each well and then put the plate back into incubator for 2h. Finally, we used Anthos 2010 Microplate Reader (Anthos Labtec Instruments GmbH, Austria) to detect the OD value at 450nm.

Statistical analysis

In this study, GraphPad Prism 8.0 (La Jolla, CA, USA) and SPSS 24.0 (Chicago, IL, USA) were performed to analyze the statistics. Results of each Experiment were shown as the mean ± standard deviation (SD) for three times independently. Analysis of two groups were implemented by Student's independent t test. Pearson chi-square test, logistic regression and Fisher's exact test were carried out to access the correlation of DDX27 and clinicopathological factors. The relevance between DDX27 and OCT4 was calculated by Spearman correlation analysis. The probabilities of survival were assessed by Kaplan-Meier assay. The condition of OS and DFS were assessed by Cox regression. Statistically significant probability values were defined as ≤ 0.05.

Results

Expression of DDX27 in breast cancer

Analysis based on TCGA-BRCA database showed that DDX27 was significantly high expressed in cancer whether it's a paired analysis or not ($p < 0.0001$, Fig. 1a-b). We evaluated DDX27 expression in 24 pairs of breast cancer samples via western blot assay and confirmed that DDX27 was significantly high expressed in cancer than matched normal tissue ($p < 0.0001$, Fig. 1c). Then, we performed IHC to assess the expression of DDX27 in 165 breast cancer patients and found DDX27 was expressed in nucleus (Fig. 2a-d). As a result, DDX27 was significantly highly expressed in cancer comparing to the normal breast tissue (11/40, 27.5%) and there were 101 breast cancer samples (61.2%) with high-expressed DDX27 and 64 samples (38.8%) with low expression.

Association of DDX27 expression with clinicopathological factors and prognosis

Association between DDX27 and clinicopathological characteristics were performed to access the influence of DDX27 in breast cancer patients. Univariate analysis (Table 1) suggested that DDX27 was positively associated with larger tumor size ($p = 0.0005$), positive lymph nodes ($p = 0.0008$), higher histological grade ($p = 0.0040$), higher ki-67 ($p = 0.0063$) and later TNM stage ($p < 0.0001$).

Table 1

The relationship between DDX27 expression and clinical pathology factors in breast cancer patients

Factors	Number (%)	DDX27		χ^2	*p*-value	Crude OR (95%CI)
		Low (%)	High (%)			
Age				0.253	0.6149[a]	
< 60	127 (76.97)	51 (30.91)	76 (46.06)			Reference
≥ 60	38 (23.03)	17 (10.30)	21 (12.73)		0.6151[b]	0.829 (0.399–1.722)
Tumor size				12.053	0.0005[a]	
< 3	95 (57.58)	50 (30.30)	45 (27.27)			Reference
≥ 3	70 (42.42)	18 (10.91)	52 (31.52)		0.0007[b]	3.210 (1.642–6.276)
Lymph node status				11.334	0.0008[a]	
Negative	109 (66.06)	55 (32.73)	54 (32.73)			Reference
Positive	56 (33.94)	13 (7.88)	43 (26.06)		0.0010[b]	3.369 (1.631–6.957)
ER				3.137	0.0765[a]	
Negative	54 (32.73)	17 (10.30)	37 (22.42)			Reference
Positive	111 (67.27)	51 (30.91)	60 (36.36)		0.0783[b]	0.541 (0.272–1.072)
PR				1.203	0.2726[a]	
Negative	54 (32.73)	19 (11.52)	35 (21.21)			Reference
Positive	111 (67.27)	49 (29.70)	62 (37.58)		0.2737[b]	0.687 (0.351–1.346)
HER2				0.232	0.6298[a]	
Negative	138 (83.64)	58 (35.15)	80 (48.48)			Reference
Positive						

p-value [a] came from Pearson Chi-square tests or Fisher's exact test

p-value [b] came from Logistic regression analyses

Factors	Number (%)	DDX27 Low (%)	DDX27 High (%)	χ^2	p-value	Crude OR (95%CI)
	27 (16.36)	10 (6.06)	17 (10.30)		0.6302[b]	1.232 (0.526–2.887)
Ki-67 index (%)				7.466	0.0063[a]	
< 20	34 (20.61)	21 (12.73)	13 (7.88)			Reference
≥ 20	131 (79.39)	47 (28.48)	84 (50.91)		0.0076[b]	2.887 (1.326–6.288)
Histological Grade				8.282	0.0040[a]	
I-II	127 (76.97)	60 (36.36)	67 (40.61)			Reference
III	38 (23.03)	8 (4.85)	30 (18.18)		0.0054[b]	3.358 (1.429–7.890)
TNM Stage				23.606	<0.0001[a]	
I	37 (22.42)	28 (16.97)	9 (16.97)			Reference
II	109 (66.06)	35 (21.21)	74 (44.85)		<0.0001[b]	6.489 (2.767–15.217)
III	19 (11.52)	5 (3.03)	14 (8.48)		0.0005[b]	9.333 (2.647–32.915)

p-value [a] came from Pearson Chi-square tests or Fisher's exact test

p-value [b] came from Logistic regression analyses

Bioinformatic analysis according to Kaplan-Meier plotter and Log-Rank test proved that higher expression of DDX27 was significantly relevant to worse OS in breast cancer ($p = 0.0013$, Fig. 3a). Further, we analyzed the implication of DDX27 on the prognosis in terms of different molecular types and found that the shorter OS associated with DDX27 was especially showed in Luminal B breast cancer ($p = 0.00083$) and Basal-like breast cancer ($p = 0.028$) (Fig. 3b-e). Moreover, we also found that higher expression of DDX27 could lead to a shorter OS in patients with lymph nodes metastasis compared to the patients without metastasis ($p = 0.38$ in lymph node negative and $p = 0.0081$ in lymph node positive, Fig. 3f-g). The Kaplan-Meier analysis and Log-Rank test suggested that DDX27 was positively and closely connected with shorter OS and DFS respectively (n = 165. $p = 0.0087$ for OS and $p = 0.0235$ for DFS, Fig. 3h-i). Finally, we found that DDX27 expression ($p = 0.017$), tumor size ($p = 0.0004$), lymph node status ($p < 0.0001$), histological grade ($p = 0.0004$) and TNM stage ($p < 0.0001$) were in connection with worse OS in univariate Cox regression, and multivariate analysis showed the independent factors contained

tumor size ($p = 0.032$), lymph node status ($p = 0.013$) and histological grade ($p = 0.015$) (Table 2). DDX27 expression ($p = 0.0289$), tumor size ($p = 0.0004$), lymph node status ($p < 0.0001$) and TNM stage ($p = 0.0001$) were correlated to worse DFS in univariate analysis. Multivariate analysis proved that tumor size ($p = 0.032$) and lymph node status ($p = 0.018$) were related to DFS independently (Table 3). Therefore, high expression of DDX27 was closely connected with different clinicopathological factors and resulted in a worse prognosis in breast cancer.

Table 2
Univariate and multivariate Cox regression analysis of DDX27 expression with regard to OS

Characteristics	Univariate analysis		Multivariate analysis	
	HR(95% CI)	*p*-value	HR(95% CI)	*p*-value
DDX27	4.437 (1.307−15.064)	0.017	1.363 (0.358−5.189)	0.650
Age	0.551 (0.162−1.871)	0.339		
Tumor size	9.220 (2.716−31.307)	0.0004	4.178 (1.127−15.488)	0.032
Lymph node status	7.613 (2.786−20.807)	<0.0001	4.339 (1.364−13.804)	0.013
ER	1.002 (0.404−2.483)	0.996		
PR	1.025 (0.414−2.539)	0.958		
HER2	1.603 (0.587−4.378)	0.357		
Ki-67 index (%)	1.640 (0.483−5.568)	0.428		
Histological Grade	4.805 (2.024−11.405)	0.0004	3.093 (1.243−7.698)	0.015
TNM Stage	6.045 (2.498−14.626)	<0.0001	1.296 (0.475−3.533)	0.613

Table 3
Univariate and multivariate Cox regression analysis of DDX27 expression with regard to DFS

Characteristics	Univariate analysis		Multivariate analysis	
	HR(95% CI)	*p*-value	HR(95% CI)	*p*-value
DDX27	2.420 (1.095−5.346)	0.0289	1.336 (0.575−3.108)	0.501
Age	0.692 (0.287−1.672)	0.414		
Tumor size	3.773 (1.804−7.893)	0.0004	2.426 (1.081−5.444)	0.032
Lymph node status	4.319 (2.135−8.736)	<0.0001	2.736 (1.190−6.290)	0.018
ER	1.040 (0.507−2.134)	0.914		
PR	1.429 (0.667−3.061)	0.359		
HER2	1.327 (0.578−3.049)	0.504		
Ki-67 index (%)	2.033 (0.716−5.771)	0.183		
Histological grade	1.736 (0.846−3.563)	0.132		
TNM Stage	4.181 (1.996−8.785)	0.0001	1.434 (0.602−3.421)	0.416

Table 4
Gene set enriched with DDX27 high expression

MsigDB collection	Gene set name	NES	NOM *p*-val	FDR q-val
c2.cgp.v6.2.symbols.gmt	JAIN_NFKB_SIGNALING	2.689	0.000	0.000
	DANG_MYC_TARGETS_UP	2.655	0.000	0.000
	WONG_EMBRYONIC_STEM_CELL_CORE	2.583	0.000	0.000
	WINTER_HYPOXIA_UP	2.711	0.000	0.000
	WELCSH_BRCA1_TARGETS_DN	2.798	0.000	0.000
h.all.v6.0.symbols.gmt	HALLMARK_DNA_REPAIR	2.683	0.000	0.000
	HALLMARK_P53_PATHWAY	1.778	0.000	0.000
	HALLMARK_PI3K_AKT_MTOR_SIGNALING	2.199	0.000	0.000
Abbreviations: NES, normalized enrichment score; NOM, nominal; FDR, false discovery rate.				

DDX27 promote breast cancer by enhancing stem cell-like properties

DDX27 was proved to act as a promoter in colorectal cancer by affecting the stem cell-like characteristics[22]. Aimed to excavate the influence of DDX27 on stem cell-like properties in breast

cancer, we analyzed the relevance between the expression of DDX27 and stemness biomarkers in TCGA-BRCA database. Results proved that DDX27 had positive correlation with the expression of OCT4 ($p <$ 0.0001) and SOX2 ($p = 0.0032$) (Fig. 5a-b). OCT4 is a classical biomarker correlated to breast cancer stem cells and has the expressed location in cell nucleus. In order to explore whether DDX27 affects the stem cell-like characteristics in breast cancer, we analyzed the association between DDX27 and OCT4 in 165 breast cancer patients by IHC staining and confirmed that DDX27 was positively related to the expression level of OCT4 ($p < 0.0001$, r = 0.428, Fig. 4a-c). Since our research group has proven technology on inducing and cultivating of MCF-7 MS and T47D MS, MCF-7 and T47D changed its morphology into mammospheres and grew rapidly 7–8 days later[24]. According to previous research of our group, the expression of stemness biomarkers and stem cell-like characteristics were obviously enhanced in MCF-7 MS and T47D MS[24, 25]. In our research, we confirmed that DDX27 was significantly high-expressed in MCF-7 MS and T47D MS compared to MCF-7 and T47D by western blot (Fig. 5c). To further elucidate DDX27 as a breast cancer stem cell biotarget, we transfected over-expression and negative control plasmid into MCF-7 and T47D cells and found that SOX2 and OCT4 were up-regulated in overexpression-DDX27 cells on protein levels (Fig. 5d).

We also investigated whether DDX27 impact on proliferation and migration in breast cancer cells. Results of CCK-8 assay proved that overexpression of DDX27 could improve the proliferation ability in MCF-7 and T47D cells (Fig. 5e). Transwell assay confirmed the improvement of migration ability in DDX27 overexpressed cells (Fig. 5f), and the wound-healing assay proved that cells with overexpressed DDX27 could significantly increase the wound-healing ability (Fig. 5g). All of our results suggested that DDX27 could enhance the stem cell-like properties in breast cancer.

DDX27-related signaling pathways

GSEA was carried out to investigate the potential molecular mechanisms related to DDX27 in breast cancer. The significance of enrichment was according to Normalized Enrichment Score (NES). The results of GSEA analysis indicated that DDX27 was positively related to NF-κB signaling, MYC targets, embryonic stem cell core, hypoxia, BRCA1 targets, DNA repair, p53 pathway and PI3K-AKT-mTOR signaling (Fig. 6a-h and Table 5). This analysis indicated that DDX27 might participate in various signaling pathways in breast cancer.

Discussion

Improving prognosis is always a pursuit of cancer therapy. Treatment strategies base on breast cancer molecular types has brought the effective improvement of diagnosis and therapy with better prognosis, which indicates that screening effective biomarkers is always of great significance. As we mentioned before, DDX27 was reported as a promoter and a biomarker with worse prognosis in hepatocellular carcinoma and gastrointestinal cancer[11–13]. Therefore, we analyzed the expression level of DDX27 and its influence on breast cancer in this research.

Results of our study confirmed that DDX27 was significantly high-expressed in both bioinformatics analysis and breast cancer samples. Analysis based on DDX27 expression and clinicopathological factors proved that high-expressed of DDX27 was closely associated with larger tumor size, positive lymph nodes, higher ki-67, higher histological grade and later TNM stage. A consistent conclusion that DDX27 was positively related to a worse prognosis was obtained on Kaplan-Meier plotter and breast cancer patients. Univariate analysis of OS and DFS showed that higher DDX27 expression, larger tumor size, positive lymph nodes and later TNM stage were correlated to worse prognosis, while the larger tumor size and positive lymph nodes were related to a worse prognosis in multivariate analysis. Based on our research, DDX27 is suggested to be a potential biomarker related to prognosis. Since we only analyzed 165 patients, multivariate analysis didn't get a positive result on DDX27 expression. In future, studies including more patients are required to investigate the effect of DDX27 in breast cancer or even on the basis of different molecular subtypes.

Cancer stem cells act as a crucial part in the processes of tumorigenesis, progress, migration, and therapeutic drug resistance[14–16]. DDX27 was proved to act as a promoter in tumorigenesis by impacting stem cell-like characteristics in colorectal cancer[22]. With the inspiration, we explored whether DDX27 could enhance the stem cell-like properties in breast cancer. In this research, we firstly accessed the correction between DDX27 expression and stemness biomarkers. Analysis based on TCGA-BRCA and breast cancer samples confirmed that DDX27 had a positive connection with the expression level of OCT4 significantly. Increased expression of stemness biomarkers and the enhanced abilities of proliferation and migration were shown in DDX27 over-expressed MCF-7 and T47D cells, which indicated that DDX27 could enhance the stem cell-like properties in breast cancer.

The processes of tumorigenesis, development and invasion are regulated by multifarious signaling pathways. Pathways participated in cancer stem cells might be different to normal stem cells. In this study, we analyzed DDX27-related signaling pathways for the potential molecular mechanism in breast cancer by GSEA. NF-κB pathway has a great influence on cell proliferation, inflammation, immunity and can regulate the biological behaviors of breast cancer stem cells[26, 27]. Enhancement of NF-κB pathway perhaps indicates that DDX27 might facilitate the processes of Epithelial-Mesenchymal Transition (EMT) and have effects on the therapeutic drug resistance in breast cancer[28]. Interestingly, DDX27 was reported to increase cancer progress and metastasis by regulating NF-κB in colorectal cancer[12]. MYC is known as a crucial factor in cancer stem cells. Overexpression of MYC might induce the ability of self-renewal and multidirectional differentiation in breast cancer stem cells[29]. Connection between DDX27 expression and hypoxia pathway might relate to oxidative stress responses in tumorigenesis in breast cancer. DDX27 correlated to BRCA1 targets and DNA repair, which suggested that DDX27 might take part in the process of genetic mutation. On the basis of p53 pathway, DDX27 might participate in breast cancer development by regulating the abilities of proliferation and migration. PI3K-AKT-mTOR pathway was observed to influence stemness and acted as a therapeutic target in breast cancer. Our analysis also found that DDX27 was relevant to PI3K-AKT-mTOR pathway, which might provide new ideas for treatment of breast cancer. To sum up, DDX27 might take part in the evaluation of breast cancer via multiple pathways, but the precise regulation mechanism is still unclear. In future research, it is necessary to carry

out the mechanistic investigations to explore the influence of DDX27 on the biological properties in breast cancer.

Conclusions

In conclusion, DDX27 was significantly high-expressed in cancer contrasted to the normal breast tissue. According to our research, the expression level of DDX27 had a closely association with larger tumor, positive lymph nodes, higher histological grade, later TNM stage and a worse prognosis. Our study suggested that DDX27 promoted the evaluation of breast cancer by influence stem cell-like properties and the exploration of DDX27-related signaling pathways has the potential significance on figuring out the molecular mechanism of breast cancer development. In short, DDX27 is bound up with the poor prognosis by enhancing stem cell-like properties and may become a potential therapeutic target in breast cancer.

List Of Abbreviations

DDX27: DEAD-box helicase 27

IHC: Immunohistochemistry

GSEA: Gene set enrichment analysis

OS: Overall survival

DFS: Disease free survival

TCGA-BRCA: The Cancer Genome Atlas-Brest Cancer

ER: estrogen receptor

PR: progesterone receptor

HER2: human epidermal growth factor receptor 2

PBS: phosphate-buffered saline

BSA: bovine serum albumin

DAB: diaminobenzidine

CCK-8: Cell Counting Kit-8

NES: Normalized Enrichment Score

EMT: Epithelial-Mesenchymal Transition

Declarations

Ethics approval and consent to participate: The study was supported by Ethics Review Committee of the First Affiliated Hospital of China Medical University (AF - SOP - 07 - 1.1 - 01) and they claimed to approve this study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the Helsinki declaration and its later amendments or comparable ethical standards. The plans for the use of the data were approved. The need of written informed consent from the patients was waived due to the retrospective nature of the study.

Consent for publication: Not applicable.

Authors' Contributions: SL, SC and FJ designed the study. FJ helped in implementing the research. SC helped in reviewing the manuscript. SL did the experiment based on fresh breast tissue and breast cancer cell lines, also wrote the main manuscript. JFM collected breast cancer sections and performed on IHC. AZ collected breast cancer tissue after surgery and contributed to statistical analysis of laboratorial experiment. XYS did the work of bioinformatics analysis. All authors have read and approved the final manuscript.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021.

2. Rocak S, Linder P. DEAD-box proteins: the driving forces behind RNA metabolism. Nat Rev Mol Cell Biol. 2004;5(3):232-41.

3. Linder P, Jankowsky E. From unwinding to clamping - the DEAD box RNA helicase family. Nat Rev Mol Cell Biol. 2011;12(8):505-16.

4. Song H, Ji X. The mechanism of RNA duplex recognition and unwinding by DEAD-box helicase DDX3X. Nat Commun. 2019;10(1):3085.

5. Hondele M, Sachdev R, Heinrich S, Wang J, Vallotton P, Fontoura BMA, et al. DEAD-box ATPases are global regulators of phase-separated organelles. Nature. 2019;573(7772):144-8.

6. Tsai TY, Wang WT, Li HK, Chen WJ, Tsai YH, Chao CH, et al. RNA helicase DDX3 maintains lipid homeostasis through upregulation of the microsomal triglyceride transfer protein by interacting with HNF4 and SHP. Sci Rep. 2017;7:41452.

7. Li Z, Zhou M, Cai Z, Liu H, Zhong W, Hao Q, et al. RNA-binding protein DDX1 is responsible for fatty acid-mediated repression of insulin translation. Nucleic Acids Res. 2018;46(22):12052-66.

8. Sarkar M, Ghosh MK. DEAD box RNA helicases: crucial regulators of gene expression and oncogenesis. Front Biosci (Landmark Ed). 2016;21:225-50.

9. Kellner M, Rohrmoser M, Forne I, Voss K, Burger K, Muhl B, et al. DEAD-box helicase DDX27 regulates 3' end formation of ribosomal 47S RNA and stably associates with the PeBoW-complex. Exp Cell Res. 2015;334(1):146-59.

10. Bennett AH, O'Donohue MF, Gundry SR, Chan AT, Widrick J, Draper I, et al. RNA helicase, DDX27 regulates skeletal muscle growth and regeneration by modulation of translational processes. PLoS Genet. 2018;14(3):e1007226.

11. Tsukamoto Y, Fumoto S, Noguchi T, Yanagihara K, Hirashita Y, Nakada C, et al. Expression of DDX27 contributes to colony-forming ability of gastric cancer cells and correlates with poor prognosis in gastric cancer. Am J Cancer Res. 2015;5(10):2998-3014.

12. Tang J, Chen H, Wong CC, Liu D, Li T, Wang X, et al. DEAD-box helicase 27 promotes colorectal cancer growth and metastasis and predicts poor survival in CRC patients. Oncogene. 2018;37(22):3006-21.

13. Wang D, Zhu ZZ, Jiang H, Zhu J, Cong WM, Wen BJ, et al. Multiple genes identified as targets for 20q13.12-13.33 gain contributing to unfavorable clinical outcomes in patients with hepatocellular carcinoma. Hepatol Int. 2015;9(3):438-46.

14. Miyoshi N, Mizushima T, Doki Y, Mori M. Cancer stem cells in relation to treatment. Jpn J Clin Oncol. 2019;49(3):232-7.

15. Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, et al. Cancer stem cells--perspectives on current status and future directions: AACR Workshop on cancer stem cells. Cancer Res. 2006;66(19):9339-44.

16. Pece S, Tosoni D, Confalonieri S, Mazzarol G, Vecchi M, Ronzoni S, et al. Biological and molecular heterogeneity of breast cancers correlates with their cancer stem cell content. Cell. 2010;140(1):62-73.

17. D'Oronzo S, Silvestris E, Lovero D, Cafforio P, Duda L, Cormio G, et al. DEAD-Box Helicase 4 (Ddx4)(+) Stem Cells Sustain Tumor Progression in Non-Serous Ovarian Cancers. Int J Mol Sci. 2020;21(17).

18. Zhou F, Liu Y, Rohde C, Pauli C, Gerloff D, Kohn M, et al. AML1-ETO requires enhanced C/D box snoRNA/RNP formation to induce self-renewal and leukaemia. Nat Cell Biol. 2017;19(7):844-55.

19. Santoriello C, Sporrij A, Yang S, Flynn RA, Henriques T, Dorjsuren B, et al. RNA helicase DDX21 mediates nucleotide stress responses in neural crest and melanoma cells. Nat Cell Biol. 2020;22(4):372-9.

20. Alqahtani H, Gopal K, Gupta N, Jung K, Alshareef A, Ye X, et al. DDX17 (P72), a Sox2 binding partner, promotes stem-like features conferred by Sox2 in a small cell population in estrogen receptor-positive breast cancer. Cell Signal. 2016;28(2):42-50.

21. Kao SH, Cheng WC, Wang YT, Wu HT, Yeh HY, Chen YJ, et al. Regulation of miRNA Biogenesis and Histone Modification by K63-Polyubiquitinated DDX17 Controls Cancer Stem-like Features. Cancer Res. 2019;79(10):2549-63.

22. Yang C, Li D, Bai Y, Song S, Yan P, Wu R, et al. DEAD-box helicase 27 plays a tumor-promoter role by regulating the stem cell-like activity of human colorectal cancer cells. Onco Targets Ther. 2019;12:233-41.

23. Lanczky A, Nagy A, Bottai G, Munkacsy G, Szabo A, Santarpia L, et al. miRpower: a web-tool to validate survival-associated miRNAs utilizing expression data from 2178 breast cancer patients. Breast Cancer Res Treat. 2016;160(3):439-46.

24. Yan Y, Liu F, Han L, Zhao L, Chen J, Olopade OI, et al. HIF-2alpha promotes conversion to a stem cell phenotype and induces chemoresistance in breast cancer cells by activating Wnt and Notch pathways. J Exp Clin Cancer Res. 2018;37(1):256.

25. Zheng A, Song X, Zhang L, Zhao L, Mao X, Wei M, et al. Long non-coding RNA LUCAT1/miR-5582-3p/TCF7L2 axis regulates breast cancer stemness via Wnt/beta-catenin pathway. J Exp Clin Cancer Res. 2019;38(1):305.

26. Hayden MS, Ghosh S. Shared principles in NF-kappaB signaling. Cell. 2008;132(3):344-62.

27. Shostak K, Chariot A. NF-kappaB, stem cells and breast cancer: the links get stronger. Breast Cancer Res. 2011;13(4):214.

28. Huber MA, Azoitei N, Baumann B, Grunert S, Sommer A, Pehamberger H, et al. NF-kappaB is essential for epithelial-mesenchymal transition and metastasis in a model of breast cancer progression. J Clin Invest. 2004;114(4):569-81.

29. Yoshida GJ. Emerging roles of Myc in stem cell biology and novel tumor therapies. J Exp Clin Cancer Res. 2018;37(1):173.
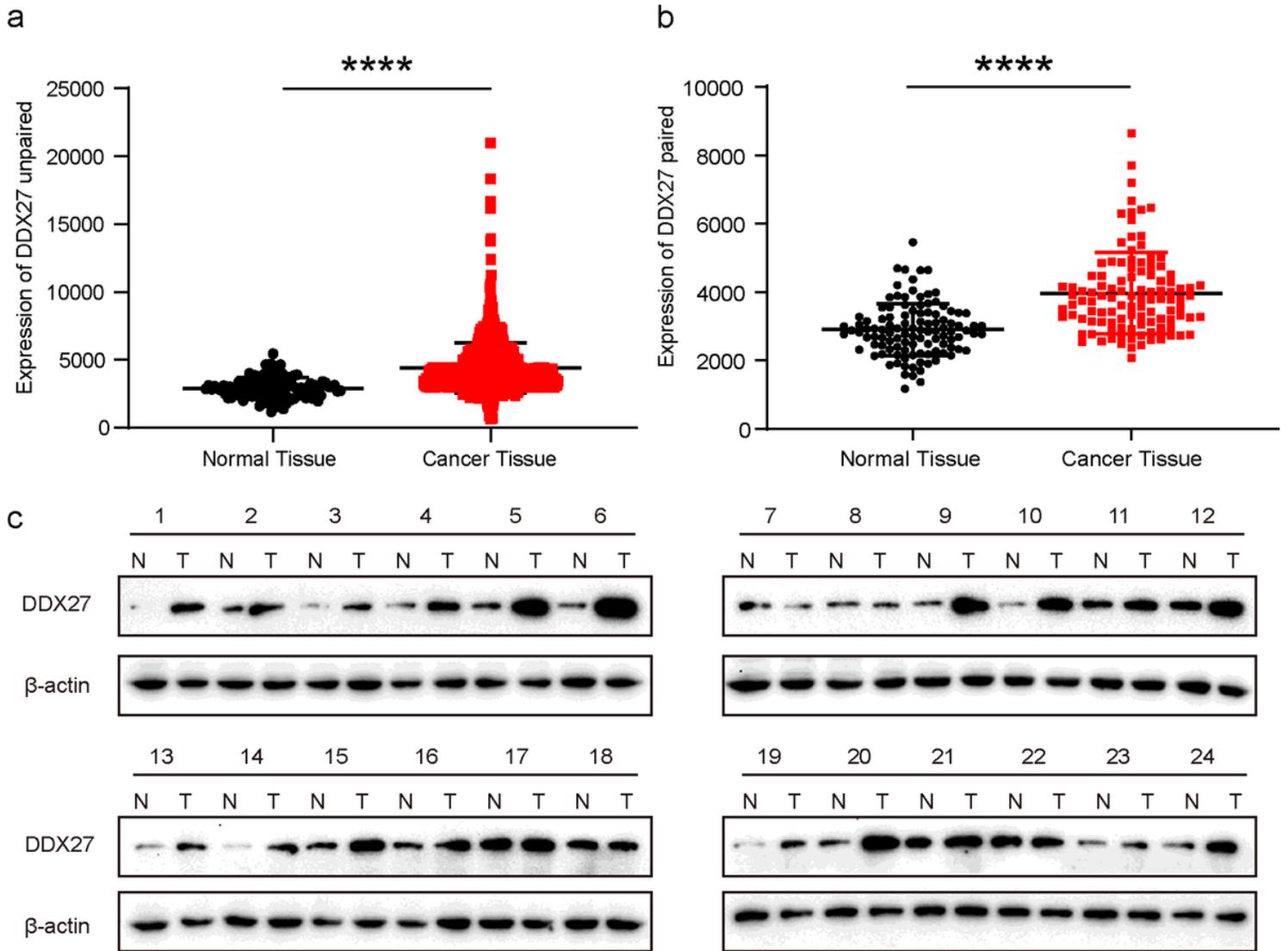
Figures

Figure 1

Expression of DDX27 in breast cancer. (a) (b) DDX27 was significantly high-expressed in breast cancer than normal tissue in TCGA-BRCA database (p < 0.0001). (c) DDX27 was high-expressed in breast cancer compared with matched normal tissue (p < 0.0001). *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.
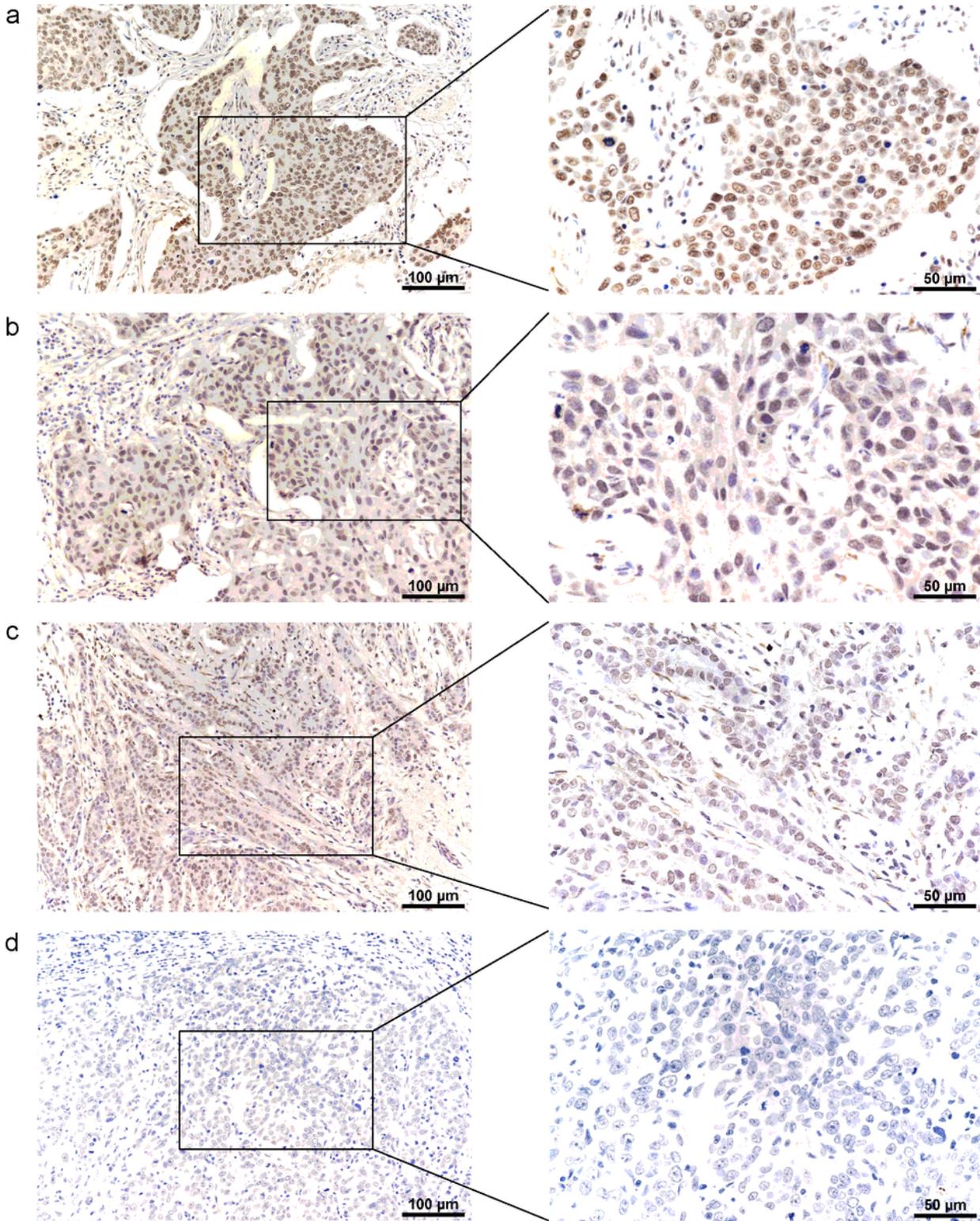
Figure 2

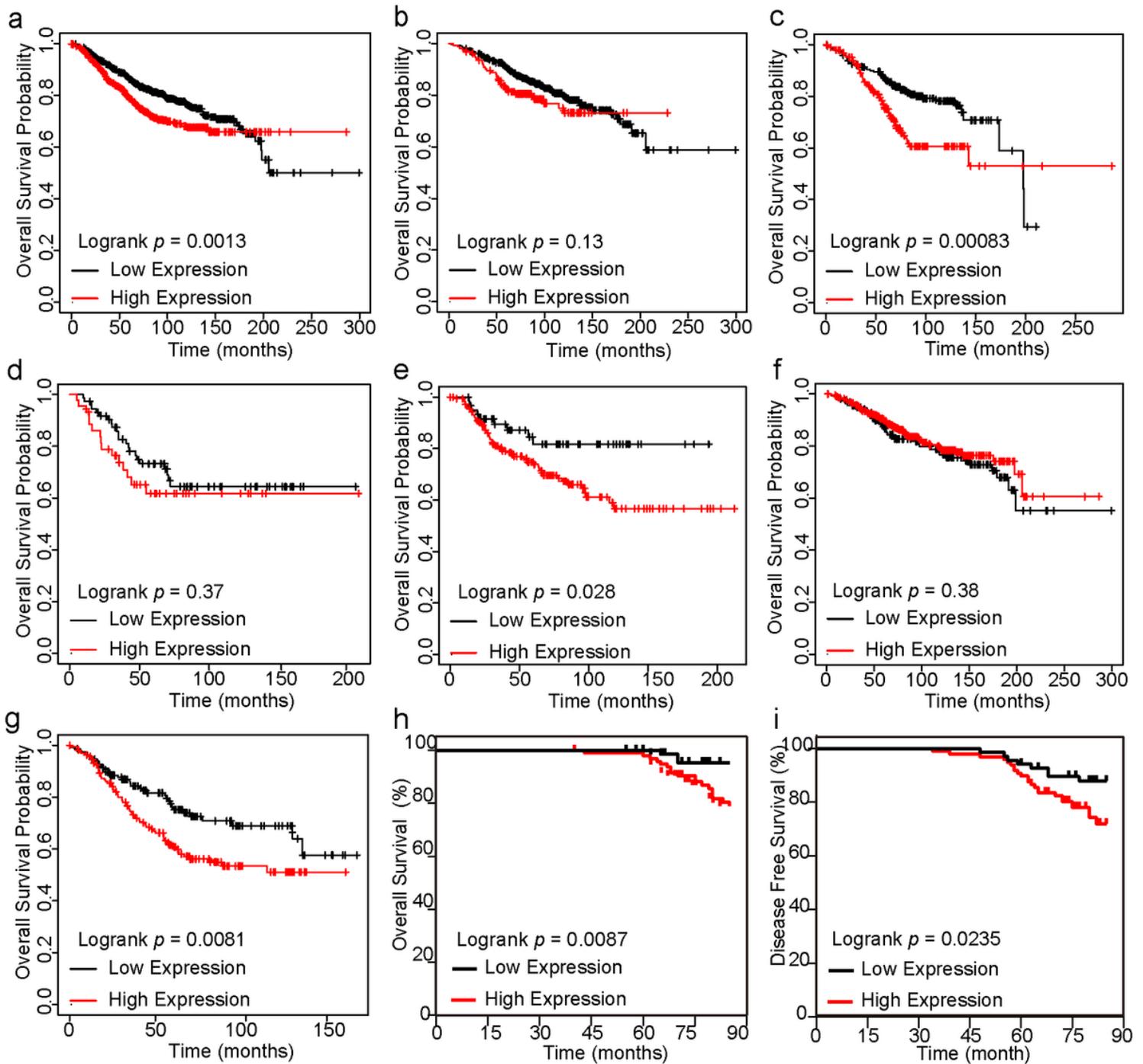IHC staining of DDX27. (a) Deep staining. (b) Medium staining. (c) Light staining. (d) Negative staining.

Figure 3

Association between DDX27 expression and prognosis. (a) High DDX27 expression was related to shorter OS in all breast cancer patients in Kaplan-Meier plotter (p = 0.0013). (b) High DDX27 expression was related to shorter OS in Luminal A breast cancer patients in Kaplan-Meier plotter (p = 0.13). (c) High DDX27 expression was related to shorter OS in Luminal B breast cancer patients in Kaplan-Meier plotter (p = 0.00083). (d) High DDX27 expression was related to shorter OS in HER2 breast cancer patients in Kaplan-Meier plotter (p = 0.37). (e) High DDX27 expression was related to shorter OS in Basal-like breast cancer patients in Kaplan-Meier plotter (p = 0.028). (f) High DDX27 expression was not related to shorter OS in lymph node negative breast cancer patients in Kaplan-Meier plotter (p = 0.38). (g) High DDX27

expression was related to shorter OS in lymph node positive breast cancer patients in Kaplan-Meier plotter (p = 0.0081). (h) High expression of DDX27 was significantly associated with a shorter OS in 165 patients (p = 0.0087). (i) High expression of DDX27 was significantly associated with a shorter DFS in 165 patients (p = 0.0235).
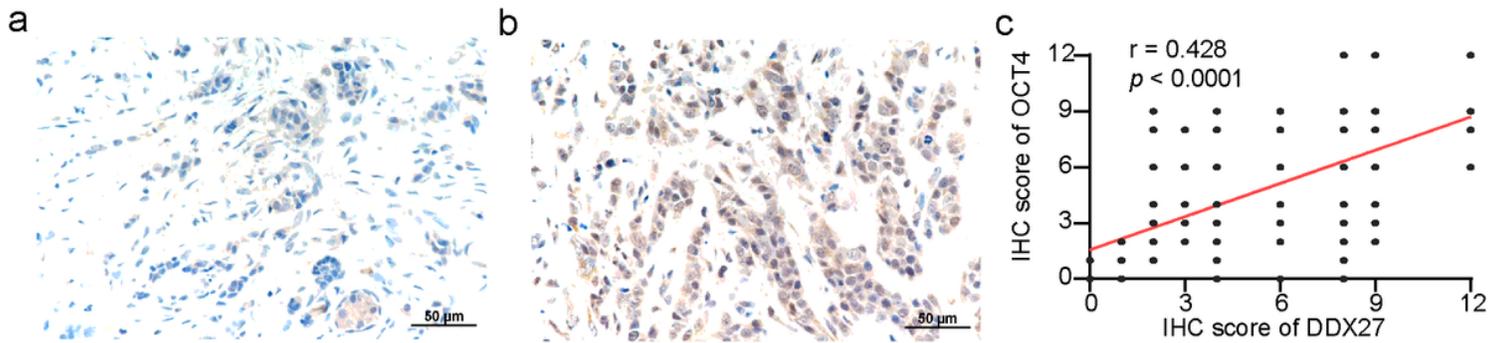

Figure 4

Relationship between DDX27 and OCT4 by IHC staining. (a) Low expression of OCT4. (b) High expression of OCT4. (c) Correlation between DDX27 and OCT4 expression by IHC staining (Spearman p < 0.0001, r = 0.428).
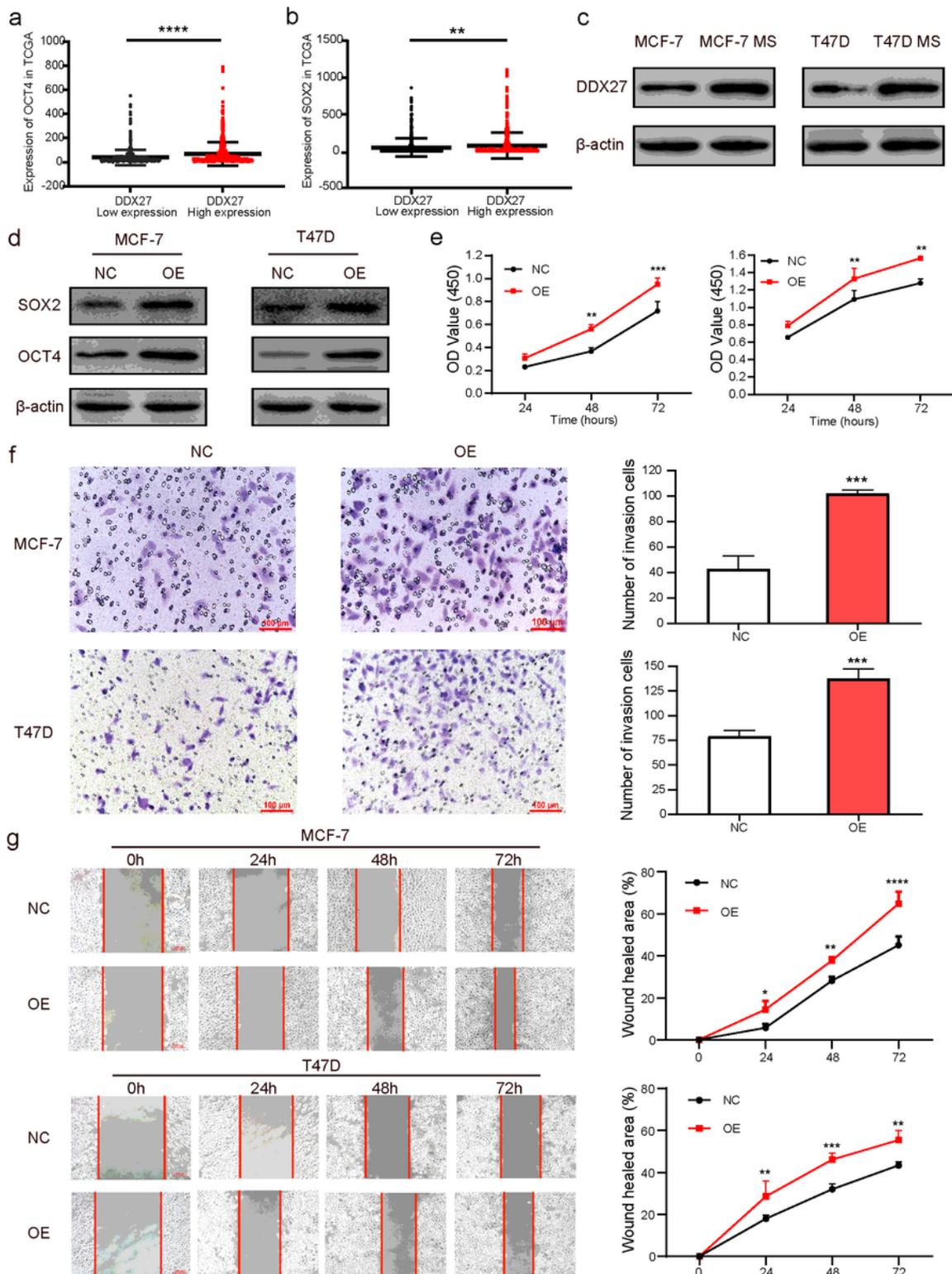
Figure 5

DDX27 promotes the stem cell-like properties of breast cancer cells. (a) (b) High expression of DDX27 was positively related to OCT4 and SOX2. (c) DDX27 was significantly high expressed in MCF-7 MS and T47D MS. (d) Proliferation abilities of breast cancer cells by CCK-8 assay. (e) Migration abilities of breast cancer cells by the Transwell assay. (f) Migration abilities of breast cancer cells by the wound-healing assay. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$.
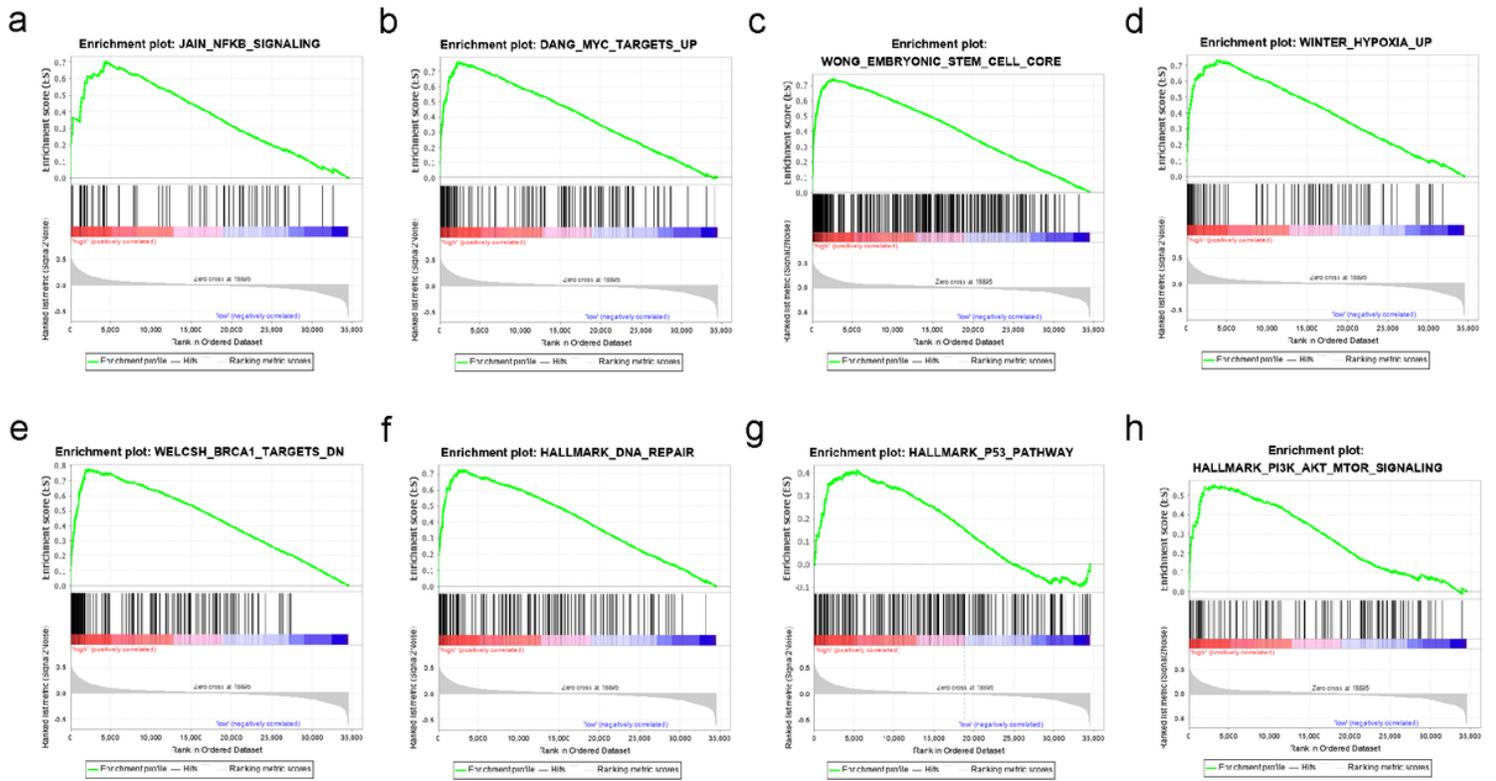
Figure 6

DDX27-related signaling pathways from Gene Set Enrichment Analysis (GSEA). (a) NF-κB pathway. (b) MYC targets. (c) Embryonic stem cell core. (d) Hypoxia. (e) BRCA1 targets. (f) DNA repair. (g) p53 pathway. (h) PI3K-AKT-mTOR pathway.