Original Article

The expression and potential mechanism of *EGFR* and *EZH2* in breast cancer

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Background: The purpose of our research was to investigate the expression of epidermal growth factor receptor (*EGFR*) and zeste gene enhancer homolog 2 (*EZH2*) in breast cancer, and to explore their potential common pathways.

Methods: Western blot and quantitative real-time polymerase chain reaction (qRT-PCR) were used to detect the protein and corresponding mRNA expression of *EGFR* and *EZH2* in breast cancer tissues and benign tissues. Then, the relationship between *EGFR* and *EZH2* along with the corresponding clinicopathological parameters were also analyzed. Bioinformatics tools were applied to explore the possible common pathways.

Results: The results showed that both *EGFR* and *EZH2* protein and mRNA were highly expressed in breast cancer tissues, and there was a positive correlation between *EGFR* and *EZH2*. Moreover, we found that increased mRNA expression was correlated with lymph node metastasis and clinical stage ($P<0.05$). Furthermore, the enrichment results of co-expressed genes indicated that *EGFR* and *EZH2* may work together in the *FOXO* signaling pathway, affecting the growth and metastasis of breast cancer cells.

Conclusions: The high expression of both *EGFR* and *EZH2* mRNA in breast cancer was related to lymph node metastasis and clinical staging. The *FOXO* signaling pathway may be their common signaling pathway that affects tumor cell invasion and metastasis.

Keywords: Breast cancer; epidermal growth factor receptor (*EGFR*); zeste gene enhancer homolog 2 (*EZH2*); infiltration and metastasis; *FOXO* signaling pathway

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Introduction

Breast cancer is one of the most common tumors in women worldwide, with an incidence rate of about 16% of all tumors, and its related mortality ranks first among female malignant tumors (1). In China, women who died from breast cancer alone in 2014 accounted for 7.82% of the total deaths from female-related cancers (2). In fact, the average age at Chinese women diagnosed with breast cancer is 45–55 years old, which is lower than the average of women in western countries, because of the application of assisted reproductive technology, birth patterns, lifestyle, environmental factors and so on (3,4). Epidermal growth factor receptor (*EGFR*) activation can induce phosphorylation of ERBB dimer partners, thereby

affecting intracellular pathways such as RAS, RAF, MEK, ERK, PI3K, AKT, TOR, and Src kinase, and transcription factors such as STAT (5). At present, studies have proven that *EGFR* plays an important role in the occurrence and development of bladder cancer, prostate cancer, and other malignant tumors (6-8). Breast cancer is also involved in the same procedure (9). Zeste gene enhancer homolog 2 (*EZH2*) is a member of the polycomb group (PcG) family. A study demonstrated that *EZH2* can affect the stability of ribosomal DNA, thereby promoting the growth and metastasis of breast cancer cells (10). The inhibition of the catalytic activity of *EZH2* can target the metastatic subtype of triple negative breast cancer (TNBC) (11). Although *EGFR* and *EZH2* have the similar effects, there are few studies which focus on the effect of *EGFR* and *EZH2* co-expression on breast cancer. Additionally, whether *EGFR* and *EZH2* have a common mechanism of action in the process of breast cancer is still unclear. This study analyzed the expression of *EGFR* and *EZH2* at the protein and mRNA levels, along with their association with clinicopathological factors. The possible common signaling pathways were also investigated, as well as the mechanisms of invasion and metastasis of breast cancer. We present the following article in accordance with the REMARK reporting checklist (available at https://dx.doi.org/10.21037/gs-21-505).

Methods

Patients

A total of 120 cases of cancer tissues were collected from patients who were diagnosed with invasive breast cancer in the Department of Gastrointestinal and Gland Surgery of the First Affiliated Hospital of Guangxi Medical University from September 2017 to September 2018. The patients were aged from 29 to 74 years old, with an average age of 50.26±10.63 years old. At the same time, 30 breast specimens that were obtained by Mammotome surgery and whose pathological results were benign lesions were collected. Patients were aged from 21 to 68 years old, with an average age of 41.41±11.31 years old. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional ethics board of First Affiliated Hospital of Guangxi Medical University [No. 2021(KY-E-038)] and informed consent was taken from all the patients.

Western blot analysis

The total protein was extracted using radioimmunoprecipitation (RIPA) protein lysate (Solarbio, Beijing, China). The protein concentration was determined by the BCA method. The protein samples were electrophoresed on a polyacrylamide gel and then transferred to a polyvinylidene fluoride (PVDF) membrane (Solarbio, Beijing, China). After blocking with 5% skim milk, the membrane was incubated with the primary antibody (Cell Signal Technology, Danvers, MA, USA) at 4 ℃ overnight. After washing with Tris buffered saline Tween (TBST), the membrane was incubated with the secondary antibody. The developer solution was added and allowed to soak for 2 minutes, then was placed in the analyzer (BIO-RAD, Hercules, CA, USA) for development.

Quantitative real-time polymerase chain reaction (qRT-PCR)

An RNA extraction kit (TAKARA, Dalian, China) was used to extract total RNA from tissues, then an ultraviolet spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the RNA concentration. Subsequently, cDNA was synthesized according to the instructions of the reverse transcription kit (TAKARA). The reaction conditions of qRT-PCR were pre-denaturation at 95 ℃ for 30 s, denaturation at 95 ℃ for 5 s, and annealing at 60 ℃ for 34 s, for a total of 40 cycles. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression level.

The primer sequences used in this experiment were as follows:

GAPDH: upstream primer: 5'-GCA CCG TCA AGG CTG AGA AC-3'; downstream primer: 5'-TGG TGA AGA CGC CAG TGG A-3'.

EZH2: upstream primer: 5'-AAA TCA GAG TAC ATG CGA CTG A-3'; downstream primer: 5'-GTA TCC TTC GCT GTT TCC ATT C-3'.

EGFR: upstream primer: 5'-ACC CAT ATG TAC CAT CGA TGT C-3'; downstream primer: 5'-GAA TTC GAT GAT CAA CTC ACG G-3'.

Bioinformatics tool analysis

Using the online database cBioPortal (12), two datasets (METABRIC, Nature 2012 & Nat Commun 2016, 2,509 samples; TCGA, PanCancer Atlas, 1,084 samples) were used to select the first 400 genes co-expressed with *EGFR* and *EZH2*. The Spearman correlation coefficient was set to

Table 1 Comparison of the basic characteristics between the breast cancer group and the benign breast lesion group

Parameters	Benign	Breast cancer	t	P value
Age	41.41±11.31	50.26±10.63	3.971	0.000
Height	157.38±4.56	156.22±5.78	1.516	0.132
Body weight	53.44±7.06	56.14±8.93	–1.009	0.315

Table 2 Comparison of basic characteristics in different subtypes of the breast cancer group

Parameters	LA	LB HER2(+)	HER2(+)	TNBC	F	P value
Age	48.10±10.57	51.31±13.03	52.96±8.96	49.03±8.71	1.310	0.275
Height	156.17±5.39	156.73±7.31	155.38±4.58	155.93±6.09	0.571	0.635
Body weight	56.74±8.27	55.77±7.31	56.12±8.81	56.83±9.58	0.305	0.822

LA, luminal A; LB, luminal B; TNBC, triple negative breast cancer.

Table 3 Tumor size of each subtype of the breast cancer group

Tumor size	LA	LB HER2(+)	HER2(+)	TNBC	χ^2	P value
T1	10	12	12	16	3.282	0.773
T2	19	16	15	14		
T3	1	2	3	2		

LA, luminal A; LB, luminal B; TNBC, triple negative breast cancer.

0.5020. Finally, 162 co-expressed genes of *EGFR* and 264 co-expressed genes of *EZH2* were screened out. STRING (13) online tools were used to construct a protein-protein interaction (PPI) network, and Cytoscape (14) software was applied to screen out potential functional modules and to analyze the biological functions.

Statistical analysis

SPSS 25.0 software was used to perform the statistical analysis. The qRT-PCR mRNA expression data was expressed as $\bar{x}$±s. Two groups of data were compared using the *t*-test, and multiple groups of data were compared using analysis of variance or the rank-sum test. Western blot results were analyzed using Image J software. The correlation between *EGFR* and *EZH2* expression was analyzed by Pearson correlation analysis, and the correlation between co-expressed genes was analyzed by the Spearman correlation test. The strength of the correlation was determined by the following criteria: 0.00–0.19 "very weak", 0.20–0.39 "weak", 0.40–0.59 "medium", 0.60–0.79 "strong", 0.80–1.0 "very strong". P value <0.05 was considered statistically significant.

Results

Patient baseline data

The comparisons between the breast cancer group and the benign breast lesions group were shown in *Table 1*. The differences between all subtypes of the breast cancer group were shown in *Table 2*. The tumor sizes of each subtype of the breast cancer group were shown in *Table 3*.

EGFR and EZH2 were highly expressed in breast cancer tissues

Western blot and qRT-PCR results showed that the protein and mRNA expression levels of *EGFR* and *EZH2* in breast cancer tissues were higher than those in benign breast lesions (*Tables 4*,*5*).

The expression levels of EGFR and EZH2 proteins in each subtype of the breast cancer group

In the 120 cases of breast cancer tissues, 55 cases were positive for *EGFR* protein expression, and the western

Table 4 Protein expression of *EGFR* and *EZH2* in benign breast tissues and breast cancer

Gene	Benign	Breast cancer	χ^2	P value
EGFR			5.011	0.025
+	7	55		
–	23	65		
EZH2			12.069	0.001
+	9	78		
–	21	42		

EGFR, epidermal growth factor receptor; *EZH2*, zeste gene enhancer homolog 2.

Table 5 mRNA expression of *EGFR* and *EZH2* in benign breast tissues and breast cancer

Gene	Benign	Breast cancer	t	P value
EGFR	0.493±0.294	0.684±0.317	3.105	0.026
EZH2	0.328±0.144	0.471±0.239	2.134	0.040

EGFR, epidermal growth factor receptor; *EZH2*, zeste gene enhancer homolog 2.

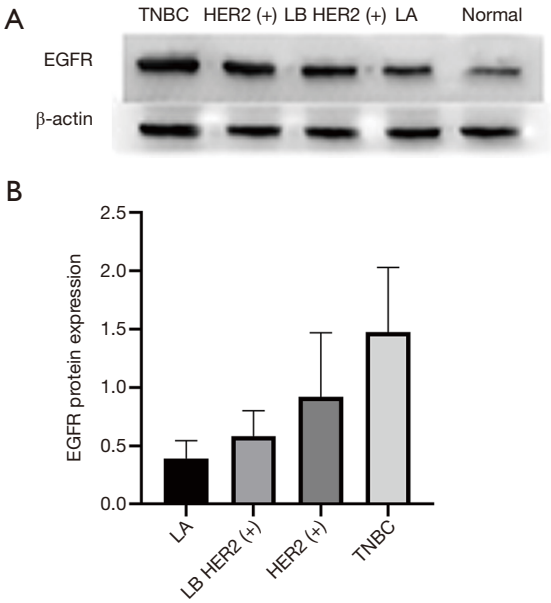

Figure 1 *EGFR* protein expression in different molecular subtypes of breast cancer. (A) Western blot results of each subtype of breast cancer. (B) *EGFR* protein expression of each subtype of breast cancer. *EGFR*, epidermal growth factor receptor; LA, luminal A; LB, luminal B; TNBC, triple negative breast cancer.

blot results are shown in *Figure 1A*. In the positive group, the expression levels of *EGFR* protein in luminal A (LA), luminal B (LB) HER2(+), HER2(+), and TNBC were 0.392±0.152, 0.584±0.217, 0.922±0.547, and 1.474±0.556, respectively (*Figure 1B*). The *EGFR* expression of TNBC was significantly different from the LA and LB HER2(+) types (P<0.05), while there was no significant difference among the other subtypes.

Among the 120 breast cancer tissues, 78 cases were positive for *EZH2* protein expression. The western blot results are shown in *Figure 2A*. The expression levels of *EZH2* protein in LA, LB HER2(+), HER2(+), and TNBC were 0.367±0.154, 0.628±0.204, 0.659±0.227, and 1.079±0.330, respectively (*Figure 2B*). The expression of *EZH2* protein in TNBC was significantly different from other subtypes (P<0.05), and there was no significant difference between the remaining subtypes.

Expression of EGFR and EZH2 mRNA in each subtype of breast cancer

The expression levels of *EGFR* mRNA in LA, LB HER2(+), HER2(+), and TNBC were 0.429±0.350, 0.602±0.373, 0.805±0.130, and 0.902±0.098, respectively (*Figure 3A*). *EGFR* expression in TNBC was significantly different from LA type (P<0.05), and the difference between HER2(+) and LA type was also statistically significant (P<0.05). However, there was no significant difference between the other subtypes.

The expression levels of *EZH2* mRNA in LA, LB HER2(+), HER2(+), and TNBC were 0.235±0.115, 0.375±0.246, 0.559±0.251, and 0.805±0.191, respectively (*Figure 3B*). There were significant differences between TNBC, LA, and LB HER2(+) types. At the same time, there were significant differences between LA and LB HER2(+) types, but there was no significant difference between the remaining subtypes.

Relationship between EGFR, EZH2, and clinicopathological factors

We analyzed the correlations between patient clinical data and *EGFR* and EHZ2 expression. As shown in *Table 6*, the simultaneous high expression of both *EGFR* and EHZ2 mRNA was related to lymph node metastasis and clinical stage (P<0.05).

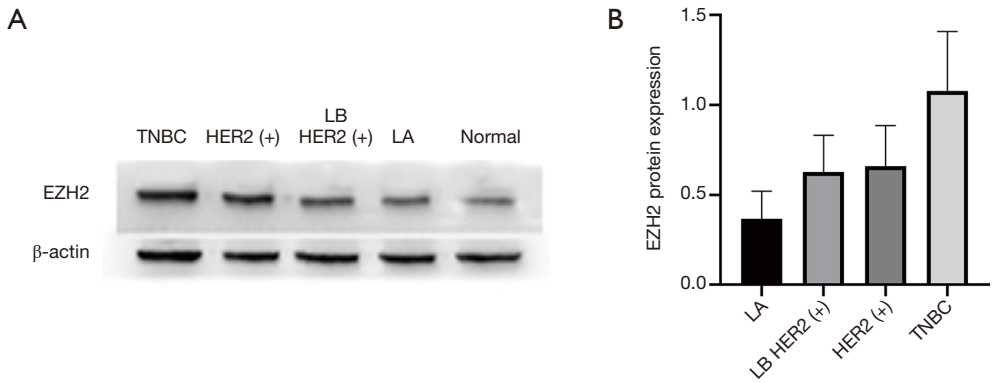

Figure 2 *EZH2* protein expression in different subtypes of breast cancer. (A) Western blot results of each subtype of breast cancer. (B) *EZH2* protein expression of each subtype of breast cancer. *EZH2*, zeste gene enhancer homolog 2; LA, luminal A; LB, luminal B; TNBC, triple negative breast cancer.

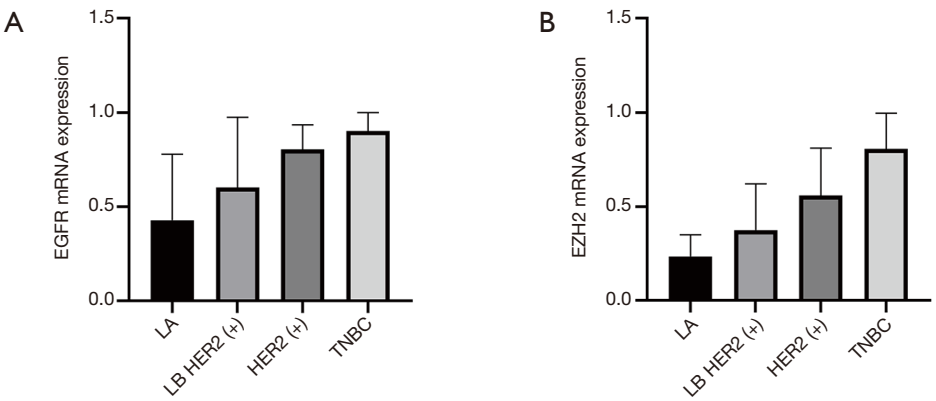

Figure 3 mRNA expression in different molecular subtypes of breast cancer. (A) *EGFR* mRNA expression of each subtype of breast cancer. (B) *EZH2* mRNA expression of each subtype of breast cancer. *EGFR*, epidermal growth factor receptor; *EZH2*, zeste gene enhancer homolog 2; LA, luminal A; LB, luminal B; TNBC, triple negative breast cancer.

Screening of co-expressed genes and establishment of the PPI network

PPI network analysis of EGFR

STRING and Cytoscape software were used to establish a PPI network of co-expressed genes. The MCODE plug-in was used to filter out the main functional modules in the network (*Figure 4A*, indicated in yellow). Meanwhile, the cytoHubba plug-in was used to screen out the top 10 genes [top 10 nodes ranked by maximal clique centrality (MCC)] (*Figure 4B*). These genes were consistent with the enrichment of functional modules screened by MCODE.

The hub genes were enriched by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis through the STRING database. The results showed that these genes were involved in the biological processes of keratinization, epithelial development, epithelial cell differentiation, estrogen signaling pathways, breast cancer, prolactin signaling pathways, and acute granulocytes. The complete KEGG enrichment was shown in Table S1.

PPI network analysis of EZH2

STRING and Cytoscape software were used to establish the PPI network of co-expressed genes. The MCODE plug-in and cytoHubba plug-in were used to filter out the main functional modules in the network (*Figure 5A*, indicated in yellow) and the top 10 genes (top 10 nodes ranked by MCC) (*Figure 5B*), respectively. The results of

Table 6 Relationship between *EGFR* and *EZH2* expression and clinicopathological factors

Clinical or pathological indicators	N	*EGFR*		*EHZ2*	
		Relative expression	P value	Relative expression	P value
Age (years)			0.199		0.645
<40	21	0.537±0.280		0.582±0.346	
40–59	71	0.715±0.263		0.526±0.324	
≥60	28	0.604±0.266		0.429±0.201	
Menopausal state			0.962		0.494
Not menopausal	65	0.651±0.280		0.478±0.290	
Menopausal	55	0.675±0.257		0.574±0.317	
T stage			0.623		0.217
T1	47	0.668±0.250		0.605±0.278	
T2	64	0.670±0.297		0.492±0.335	
T3	9	0.608±0.255		0.368±0.169	
Lymph node			0.005*		0.024*
N0	40	0.473±0.282		0.344±0.279	
N1	44	0.618±0.272		0.461±0.180	
N2	20	0.750±0.096		0.552±0.140	
N ≥3	16	0.865±0.099		0.721±0.313	
Clinical stage			0.009*		0.013*
I	25	0.361±0.221		0.243±0.226	
II	59	0.560±0.230		0.353±0.153	
III	36	0.768±0.123		0.724±0.317	
Pathological grade			0.405		0.154
I	15	0.816±0.040		0.769±0.371	
II	75	0.674±0.254		0.422±0.210	
III	30	0.592±0.318		0.610±0.373	
Ki-67			0.203		0.046*
<20%	36	0.566±0.257		0.380±0.269	
20–30%	32	0.661±0.297		0.516±0.310	
>30%	52	0.751±0.242		0.638±0.283	
P53			0.770		0.538
+	79	0.635±0.330		0.476±0.243	
–	41	0.574±0.334		0.419±0.327	

*, indicates the result is statistically significant. *EGFR*, epidermal growth factor receptor; *EZH2*, zeste gene enhancer homolog 2.

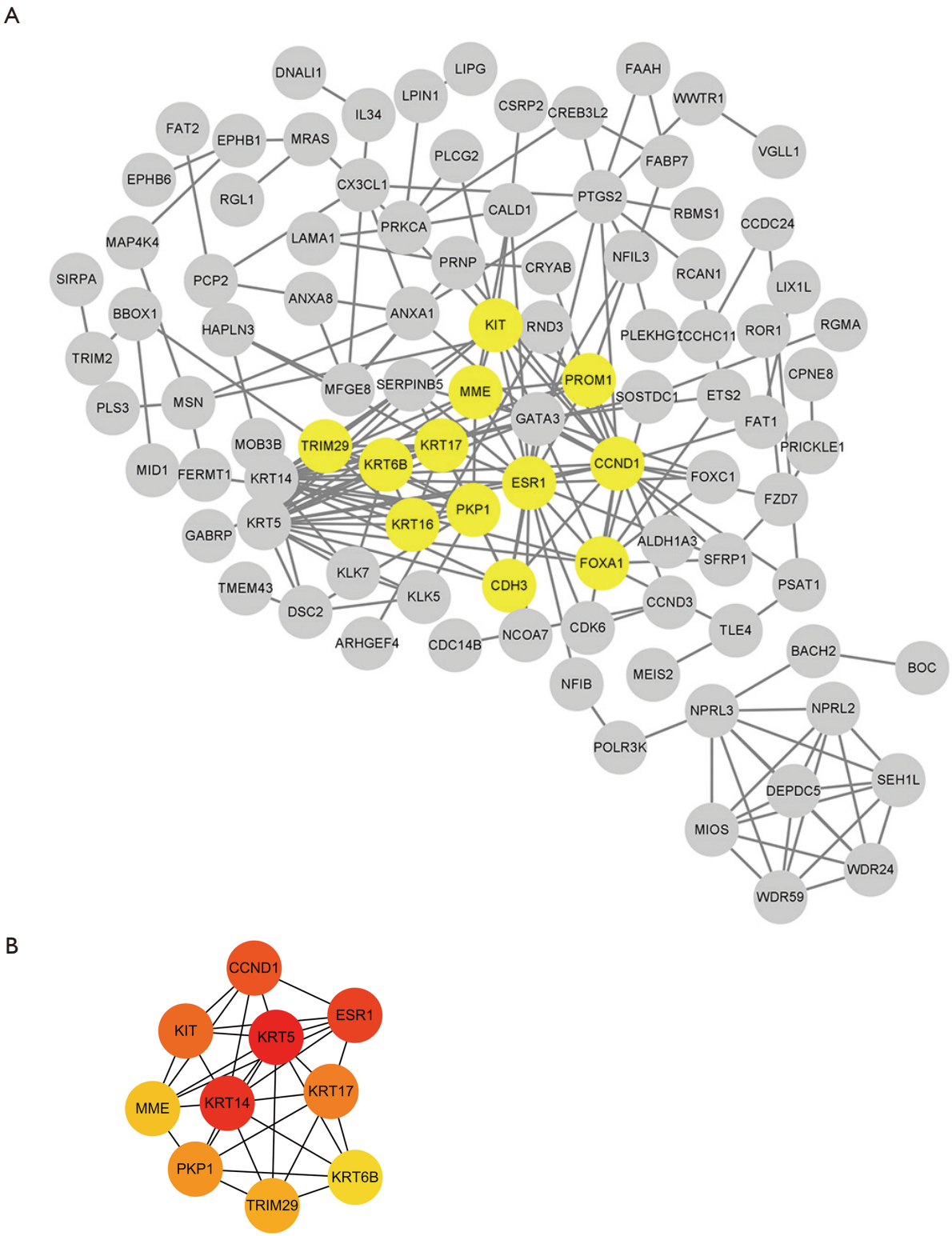

Figure 4 Co-expressed gene protein interaction network of *EGFR*. (A) The most important functional modules in the PPI network (indicated in yellow). (B) Hub genes screened by cytoHubba. *EGFR*, epidermal growth factor receptor; PPI, protein-protein interaction.

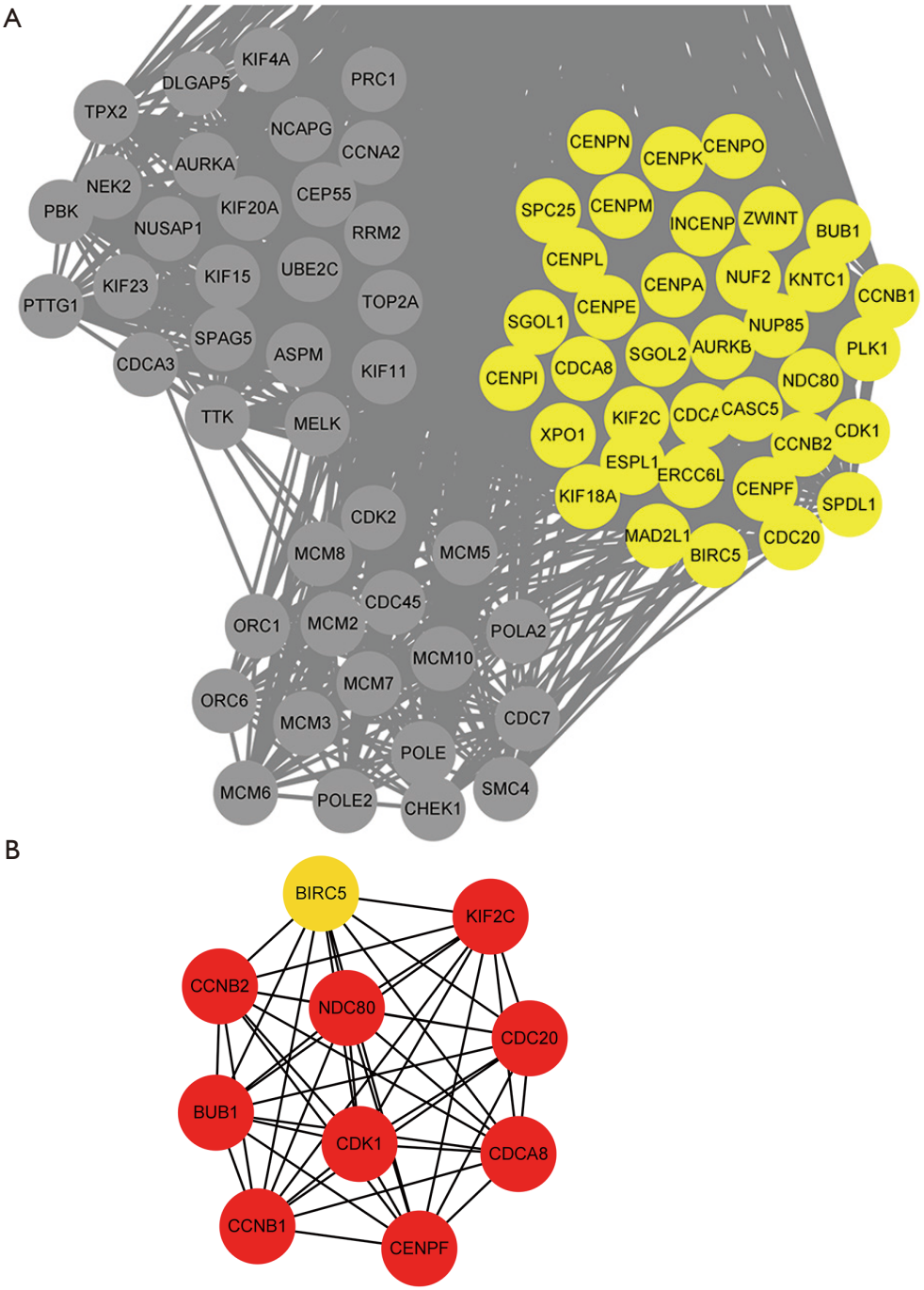

Figure 5 Co-expressed gene protein interaction network of *EZH2*. (A) The most important functional modules in the PPI network (indicated in yellow). (B) Hub genes screened by cytoHubba. *EZH2*, zeste gene enhancer homolog 2; PPI, protein-protein interaction.

the functional modules and the hub genes were consistent.

Enrichment analysis of hub genes using the STRING database showed that they were involved in cell division, mitotic cell cycle process, chromosome separation, chromosome location establishment, and other biological processes, as well as the cell cycle, p53 signaling pathway, cell senescence, *FOXO* signaling pathway, etc. The complete KEGG enrichment was shown in Table S2.

Discussion

EGFR is mainly converted into dimers by binding to ligands, and affects downstream pathways through autophosphorylation, thereby regulating cell functions. At present, there are two typical pathways known to be involved. One is the Ras-Raf-MAPK pathway, also known as the mitogen-activated protein kinase pathway, which regulates the cell cycle and cell proliferation-related functions through the activation of MAPK, ERK1, and ERK2 kinases. A research pointed out that it plays an important role in the metastasis of breast cancer cells (15). The other is the PI3K-AKT-mTOR pathway, which mainly regulates the anti-apoptotic-related responses of cells by activating serine protein kinase (16). This pathway is also a typical pathway involved in the development of HER2 overexpression and TNBC. Inhibition of this pathway can improve the prognosis of patients (17). In this study, the expression of *EGFR* in TNBC and HER2-overexpressed breast cancer was higher than that in LA breast cancer, which was consistent with the results of Su *et al.* (18). Hub gene enrichment analysis results showed that these genes were enriched in the estrogen signaling pathway, breast cancer, and prolactin signaling pathway, indicating that *EGFR* may be involved in the negative transformation processes of TNBC-related receptor targets.

EZH2 mainly acts as a transcriptional inhibitor by catalyzing the trimethylation of lysine 27 (H3K27me3) of histone H3. The methylated H3K27me3 recruits PRC2 complexes to specific gene sites which silences the expression of target genes and promotes tumor metastasis (19). A study observed that reducing the expression of *EZH2* in the cytoplasm by inhibiting its upstream factors can inhibit tumor metastasis (20). The hub gene analysis of *EZH2* demonstrated that the above genes were mainly enriched in cell cycle-related biological processes such as mitosis, nuclear division, and chromosome separation, indicating that *EZH2* may be involved in the progression of breast cancer by affecting the proliferation potential and clonality of cancer cells. In this study, the expression of *EZH2* protein in TNBC was significantly higher than in other subtypes. Nie *et al.* (21) found that the inhibition of *EZH2* and cyclin-dependent kinase 2 (CKD2) could re-express the estrogen receptor of TNBC tumor cells, thereby enabling the use of tamoxifen for endocrine therapy, which suggests that *EZH2* may also participate in the transformation process of TNBC.

The hub genes of *EZH2* were enriched in the *FOXO* signaling pathway, and the *FOXO* signaling pathway is one of the downstream pathways in which MAPK and PI3K work together. It acts on a variety of biological metabolic processes and key cancer metabolic pathways to affect tumor cell metabolism (22). A study pointed out that the up-regulation of *FOXO*3A, a member of the *FOXO* family, promotes angiogenesis, thereby enhancing the metastatic invasiveness of breast cancer cells (23). The *FOXO* family also plays an important role in the metastasis of many cancers (24-26). One study noted that the activation or inhibition of *FOXO* inhibited the growth of metastatic tumor cells (27), which is different from the role of *FOXO* as a tumor suppressor gene in traditional cognition, indicating that a certain amount of intracellular expression of *FOXO* may be necessary to maintain tumor cell survival. In this study, the protein and mRNA expression of *EGFR* and *EZH2* were positively correlated. It may be that the regulatory effects of *EGFR* and *EZH2* converge in the downstream *FOXO* pathway. The high expression of the two in TNBC may be related to the high proliferation and metastasis of TNBC. However, only high-level mRNA expression of *EGFR* and *EZH2* was related to prognostic factors such as lymph node metastasis and clinical staging ($P<0.05$). This indicates that other factors are involved in the regulation of the processes during protein translation, some scholars have reported examples of negative correlation between mRNA and protein expression (28). This study also has some limitations. There are not enough cases and few types, most of which are fibroadenoma of breast, the difference of mRNA expression in different types of benign breast lesions is difficult to be analyzed, large sample and further research are needed.

In short, by revealing the high expression of *EGFR* and *EZH2* in breast cancer, especially TNBC, along with the correlation with clinicopathological factors and possible common signaling pathways, it is expected to become a potential therapeutic target. High mRNA expression was closely related to lymph node metastasis and clinical staging, making it an important molecular marker for the poor prognosis of breast cancer.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/gs-21-505). The 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. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional ethics board of First Affiliated Hospital of Guangxi Medical University [No. 2021(KY-E-038)] and informed consent was taken from all the patients.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Li H, Zheng RS, Zhang SW, et al. Incidence and mortality of female breast cancer in China, 2014. Zhonghua Zhong Liu Za Zhi 2018;40:166-71.
3. Hu L, Bu Z, Huang G, et al. Assisted reproductive technology in china: results generated from data reporting system by CSRM from 2013 to 2016. Front Endocrinol (Lausanne) 2020;11:458.
4. Fan L, Strasser-Weippl K, Li JJ, et al. Breast cancer in China. Lancet Oncol 2014;15:e279-89.
5. Yarden Y, Pines G. The ERBB network: at last, cancer therapy meets systems biology. Nat Rev Cancer 2012;12:553-63.
6. Rose M, Maurer A, Wirtz J, et al. EGFR activity addiction facilitates anti-ERBB based combination treatment of squamous bladder cancer. Oncogene 2020;39:6856-70.
7. Rossini A, Giussani M, Ripamonti F, et al. Combined targeting of EGFR and HER2 against prostate cancer stem cells. Cancer Biol Ther 2020;21:463-75.
8. Talukdar S, Emdad L, Das SK, et al. EGFR: An essential receptor tyrosine kinase-regulator of cancer stem cells. Adv Cancer Res 2020;147:161-88.
9. Liang Y, Xu X, Wang T, et al. The EGFR/miR-338-3p/EYA2 axis controls breast tumor growth and lung metastasis. Cell Death Dis 2017;8:e2928.
10. Chu W, Zhang X, Qi L, et al. The EZH2-PHACTR2-AS1-ribosome axis induces genomic instability and promotes growth and metastasis in breast cancer. Cancer Res 2020;80:2737-50.
11. Yomtoubian S, Lee SB, Verma A, et al. Inhibition of EZH2 catalytic activity selectively targets a metastatic subpopulation in triple-negative breast cancer. Cell Rep 2020;30:755-70.e6.
12. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013;6:pl1.
13. Szklarczyk D, Gable AL, Lyon D, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res 2019;47:D607-13.
14. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003;13:2498-504.
15. Tang T, Zhu Q, Li X, et al. Protease Nexin I is a feedback regulator of EGF/PKC/MAPK/EGR1 signaling in breast cancer cells metastasis and stemness. Cell Death Dis 2019;10:649.
16. Lien EC, Dibble CC, Toker A. PI3K signaling in cancer: beyond AKT. Curr Opin Cell Biol 2017;45:62-71.
17. Bosch A, Li Z, Bergamaschi A, et al. PI3K inhibition results in enhanced estrogen receptor function and dependence in hormone receptor-positive breast cancer. Sci Transl Med 2015;7:283ra51.
18. Su YC, Burnouf PA, Chuang KH, et al. Conditional

internalization of PEGylated nanomedicines by PEG engagers for triple negative breast cancer therapy. Nat Commun 2017;8:15507.
19. Rybak AP, Bristow RG, Kapoor A. Prostate cancer stem cells: deciphering the origins and pathways involved in prostate tumorigenesis and aggression. Oncotarget 2015;6:1900-19.
20. Anwar T, Arellano-Garcia C, Ropa J, et al. p38-mediated phosphorylation at T367 induces EZH2 cytoplasmic localization to promote breast cancer metastasis. Nat Commun 2018;9:2801.
21. Nie L, Wei Y, Zhang F, et al. CDK2-mediated site-specific phosphorylation of EZH2 drives and maintains triple-negative breast cancer. Nat Commun 2019;10:5114.
22. Yadav RK, Chauhan AS, Zhuang L, et al. FoxO transcription factors in cancer metabolism. Semin Cancer Biol 2018;50:65-76.
23. Mishra R, Thorat D, Soundararajan G, et al. Semaphorin 3A upregulates FOXO 3a-dependent MelCAM expression leading to attenuation of breast tumor growth and angiogenesis. Oncogene 2015;34:1584-95.
24. Hou T, Li Z, Zhao Y, et al. Mechanisms controlling the anti-neoplastic functions of FoxO proteins. Semin Cancer Biol 2018;50:101-14.
25. Jiramongkol Y, Lam EW. FOXO transcription factor family in cancer and metastasis. Cancer Metastasis Rev 2020;39:681-709.
26. Ma J, Matkar S, He X, et al. FOXO family in regulating cancer and metabolism. Semin Cancer Biol 2018;50:32-41.
27. Hornsveld M, Smits LMM, Meerlo M, et al. FOXO transcription factors both suppress and support breast cancer progression. Cancer Res 2018;78:2356-69.
28. Lin X, Chai G, Wu Y, et al. RNA m6A methylation regulates the epithelial mesenchymal transition of cancer cells and translation of Snail. Nat Commun 2019;10:2065.

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Supplementary

Table S1 Enrichment analysis results of hub genes of *EGFR*

Enrichment	Name	FDR
GO biological process		
GO:0070268	Keratinization	1.48E–06
GO:0060429	Epithelial development	3.40E–05
GO:0030855	Epithelial cell differentiation	3.77E–05
GO:0045109	Intermediate filament organization	3.77E–05
GO:0048513	Animal organ development	3.77E–05
GO:0048869	Cell development	1.10E–04
GO:0007010	Cytoskeleton organization	1.50E–04
GO:0012501	Programmed cell death	2.20E–04
GO:0045110	Intermediate tow components	3.10E–04
GO molecular function		
GO:0005200	Structural components of the cytoskeleton	2.51E–05
GO:0005198	Structural molecular activity	7.30E–04
GO:0019215	Intermediate filament bondage	1.20E–03
GO:0046934	Phosphatidylinositol-4,5-bisphosphate 3-kinase activity	1.81E–02
GO cellular components		
GO:0005882	Intermediate wire	2.92E–06
GO:0045095	Keratin filaments	4.00E–04
GO:0044444	Cytoplasmic part	8.30E–03
GO:0005911	Cell connection	1.11E–02
KEGG		
hsa04915	Estrogen signaling pathway	2.20E–03
hsa05224	Breast cancer	2.20E–03
hsa04917	Prolactin signaling pathway	1.00E–02
hsa05221	Acute myeloid leukemia	1.00E–02
hsa01522	Endocrine resistance	1.19E–02
hsa04640	Hematopoietic cell lineage	1.19E–02
hsa04919	Thyroid hormone signaling pathway	1.26E–02
hsa05200	Cancer-related pathways	1.37E–02
hsa05205	Proteoglycans in cancer	2.72E–02

EGFR, epidermal growth factor receptor; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; FDR, false discovery rate.

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Table S2 Enrichment analysis results of hub genes of *EZH2*

Enrichment	Name	FDR
GO biological process		
GO:0051301	Cell division	6.89E–14
GO:1903047	Mitotic cell cycle process	1.60E–13
GO:0007059	Chromosome segregation	7.24E–12
GO:0051303	Establishment of chromosome location	8.34E–11
GO:0098813	Nuclear chromosome segregation	1.20E–10
GO:0051983	Regulation of chromosome segregation	2.77E–10
GO:0000280	Nuclear division	7.73E–10
GO:0000819	Sister chromatid separation	9.15E–10
GO:0140014	Mitotic nuclear division	1.41E–09
GO:0030071	Regulation of metaphase/late mitotic transition	1.61E–09
GO molecular function		
GO:0004693	Cyclin-dependent protein serine/threonine kinase activity	7.20E–05
GO:0035173	Histone kinase activity	1.30E–03
GO:0004674	Protein serine/threonine kinase activity	1.40E–03
GO:0140096	Catalytic activity acting on protein	3.13E–02
GO:0016740	Transferase activity	3.27E–02
GO:0008022	Protein C-terminal binding	4.10E–02
GO cellular components		
GO:0000775	Chromosome centromeric region	1.14E–10
GO:0000777	Condensed chromosome kinetochore	3.05E–10
GO:0000940	Condensed extrachromosomal kinetochore	8.41E–10
GO:0015630	Microtubule cytoskeleton	9.86E–10
GO:0044430	Cytoskeleton	1.38E–08
GO:0000942	Condensed nuclear extrachromosomal kinetochore	3.62E–08
GO:0005819	Spindle	4.12E–08
GO:0005815	Microtubule Organization Center	6.46E–08
GO:0005813	Centrosome	2.72E–07
GO:0043232	Intracellular non-membrane organelles	7.83E–07
KEGG		
hsa04110	Cell cycle	2.45E–08
hsa04114	Oocyte meiosis	2.45E–08
hsa04914	Progesterone-mediated oocyte maturation	4.85E–07
hsa04115	p53 signaling pathway	1.62E–05
hsa04218	Cell senescence	1.50E–04
hsa04068	*FOXO* signaling pathway	3.90E–03
hsa05203	Viruses cause cancer	6.50E–03
hsa05166	HTLV-I infection	1.04E–02

EZH2, zeste gene enhancer homolog 2; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; FDR, false discovery rate.

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