Expression and gene regulation network of EAF2 in cervical cancer based on data mining

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Primary research

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Abstract

Background: ELL-associated factor 2 (EAF2) plays an important role in transcription elongation and the regulation of gene expression in both mammalian cells as well as in lower eukaryotes concurrent . EAF2's depletion has been demonstrated to enhance cell proliferation and greatly increase the risk of cancer. However, little is known about the expression and function of EAF2 in cervical cancer (CC) progression. Here, we comprehensively analyzed the expression of EAF2 and its clinical outcome in CC using publicly available cancer gene expression and patient survival data through various databases.

Methods: We examined the differences of EAF2 expression between cancers and their normal tissues using the Oncomine, Gene expression Profiling Interactive Analysis 2 (GEPIA2), the Gene Expression across Normal and Tumor tissue 2 (GENT2) database and UALCAN databases. EAF2 expression was investigated from immunohistochemistry images using the Human Protein Atlas database. Copy number alterations (CNAs) and mutations of EAF2 were analyzed using cBioPortal. Kaplan–Meier analysis was used to predict the survival of EAF2 in CC. Analysis of the co-expression profile of EAF2 and the enrichment pathway of co-expression with EAF2 were revealed using LinkedOmics to explore the predicted signaling pathways. GeneMANIA visualize the gene networks and predict function of genes that GSEA identified as being enriched in CC: kinase LYN, mi-RNA133A, 133B and transcription factor OCT1.

Results: We found that the expression of EAF2 decreased with the development of CC and significant upregulation of EAF2 is positively correlated with the overall survival (OS) of CC patients. The decrease of EAF2 gene expression may be partly due to promoter methylation and CNAs with the development of CC. Besides, EAF2 expression might be strongly positively correlated with the expression of IQCB1, ILDR1 and ASTE1, and may contribute to a signaling pathway in CC.

Conclusion: Decreased EAF2 expression has negative clinical significance in the development of CC through the regulation of methylation, CNAs and related pathways. This suggests that EAF2 has potential as a therapeutic target for CC. Keywords: EAF2; cervical cancer; patient survival; clinical outcomes; cancer progression; multiomics analysis

Background

Deaths due to cancers are a growing threat to human survival [1]. Cervical cancer is one of the leading causes of cancer death in women. Globally, cervical cancer accounts for almost 12% of all female cancers, making it the fourth most common female cancer in the world [2,3]. According to the 2018 Global Cancer Statistics report, the incidence and mortality of CC account for 6.6% and 7.5% of all female cancer patients, respectively [4]. There have been improvements in the early detection and treatment of CC, which has improved patient survival. Yet, CC still causes many deaths, and the median overall survival rate of advanced cervical cancer was only 16.8 months [5]. To improve patient survival in CC, it is crucial to identify novel therapeutic targets to guide individualized treatment and predict the survival outcomes of patients with cervical cancer. Expression of genes is altered due to the accumulated genetic

alterations or epigenetic modifications in all types of cancers. The profiles of differentially expressed genes (DEGs) in each cancer tissue reflect the cancer characteristics that are closely related to patient prognosis. DEGs associated with patient survival in CC may be possible markers for early diagnosis and may be therapeutic targets. Determining this requires an understanding of the related mechanisms of cancer progression and aggressiveness.

EAF2 plays an important role in transcription elongation and the regulation of gene expression in both mammalian cells as well as in lower eukaryotes [6]. EAF2 usually interacts with the RNA polymerase II elongation factor eleven-nineteen lysine-rich in leukemia [7]. As for EAF2, its depletion has been demonstrated to enhance cell proliferation and greatly increase the risk of cancer in multiple mouse tissues, indicating that this factor is growth inhibitory and may act as a potential tumor suppressor [8-10]. The involvement of EAF2 in tumor progression has been reported in prostate, glioblastoma, colorectal, gastric cancers and leukemia [10-14]. However, little is known about the expression and function of the EAF2 in cervical cancer. To the best of our knowledge, EAF2 gene has not yet been elucidated using data mining tools. Therefore, this is the first data mining study to predict the possible role of EAF2 in cervical cancer based on publicly available gene expression and clinical data.

Given the fact that elevated expression of EAF2 in CC multiple expression datasets, we undertook a comprehensive analysis to investigate the expression pattern of the EAF2 gene and its clinical outcome in CC patients using numerous publicly available expression and patient survival datasets from various online platforms. In addition, we also analyzed genes that were co-altered with EAF2 in CC that might be relevant in EAF2-associated mechanisms in CC progression and prognosis. The collective data provide supportive evidence for the use of EAF2 as a potential prognostic biomarker of CC therapeutics.

Materials And Methods

Analysis of EAF2 expression in various cancers

The mRNA expression levels of EAF2 in various cancers and their normal tissue counterparts were analyzed using the Oncomine database (https://www.oncomine.org/resource/login.html) [15], Gene expression Profiling Interactive Analysis 2 (GEPIA2) (http://gepia.cancer-pku.cn/) [16], and the Gene Expression across Normal and Tumor tissue 2 (GENT2) database (http://gent2.appex.kr) [17]. In Oncomine database, the cut-off of p value and fold change were as following: p value: 0.01, fold change: 1.0, gene rank: 10%. In GEPIA2, EAF2 expression of tumor samples in The Cancer Genome Atlas (TCGA) were compared to combined expression data of normal adjacent mucosa in TCGA and normal healthy cervical in Genotype-Tissue Expression (GTEx). EAF2 queries were carried out with default settings to obtain their respective expression pattern in all analyses with these databases.

Analysis of EAF2 expression in cervical cancer and its normal tissue

EAF2 mRNA expression in cervical cancer and normal counterparts was examined in the Oncomine database and the UALCAN web (http://ualcan.path.uab.edu/index.html) [18]. In the Oncomine database,

the fold-change in mRNA expression in cervical tumor tissue compared to the normal tissue was obtained using the parameters of p-value < 0.01, fold-change > 1, and gene ranking in the top 10%. Normalized raw transcriptome data were subsequently analyzed to evaluate the relative expression of EAF2 in cervical cancer relative to normal cervical tissue. Expression of the EAF2 protein in cervical cancer and normal tissue was directly investigated in immunohistochemistry images retrieved from the Human Protein Atlas database [19].

EAF2 gene expression and promoter methylation analysis in each clinical characteristic with data from TCGA

EAF2 mRNA expression in each cervical cancer patient characteristic was analyzed in TCGA datasets using the UALCAN web with default settings. EAF2 mRNA expression in cancer was separately analyzed with patient characteristics of sample types, individual cancer stage, race, weight, age, nodal metastasis status and tumor histology compared to the normal cervical tissue expression. Promoter methylation was also analyzed according to sample types, individual cancer stage, tumor grade, tumor histology and nodal metastasis status in UALCAN web.

Evaluation of mutations and CNAs of the EAF2 Gene in cervical cancer

We analyzed the mutations and CNAs of the EAF2 gene using the cBioPortal web (http://www.cbioportal.org/) [20]. The frequency of the mutations was estimated from samples from two studies available for cervical in cBioPortal. Somatic CNAs were generated from RNA-seq data by the GISTIC (genomic identification of significant targets in cancer) algorithm with default settings and plotted with mRNA expression data using cBioPortal web. Correlation statisticswas performed using Graph Pad Prism 7.0. The statistical analysis between two variables was performed by unpaired t-test.

Evaluation of the relationship between EAF2 expression and patient survival in cervical cancer

The correlation between mRNA expression level of the EAF2 gene and the survival probability of cervical cancer patients was analyzed using the Kaplan-Meier plot (http://kmplot.com/analysis/) database. In brief, we input the gene name of the EAF2 into the gene symbol search box and adjusted the survival type to OS. We kept all of the default settings of the Kaplan-Meier plot database then plotted the Kaplan-Meier curve. Then it was verified by GEPIA2 database. Statically significant difference was considered when a p value < 0.05.

Profiling of genes co-expressed with EAF2

The co-expression profile of the EAF2 genes were analyzed using the LinkedOmics database (http://www.linkedomics.org/login.php) [21]. The LinkFinder module of LinkedOmics was used to study genes differentially expressed in correlation with EAF2 in the TCGA CESC cohort (n=273). Results were analyzed statistically using Pearson's correlation coefficient. The LinkFinder also created statistical plots for individual genes. All results were graphically presented in volcano plots, heat maps or scatter plots.

Gene Ontology (GO), KEGG pathway, kinase, miRNA and transcription factor enrichment analyses of EAF2 and co-expressed genes

Data from the LinkFinder results were signed and ranked, and gene set enrichment analysis (GSEA) was used to perform analyses of GO (CC: cellular component, BP: biological process and MF: molecular function), KEGG pathways, kinase-target, miRNA-target and transcription factor-target enrichment. The latter two network analyses were based on the Molecular Signa-tures Database [22]. The Link-Interpreter module of LinkedOmics performs pathway analyses of differentially expressed genes. We used GeneMANIA (http://www.genemania.org) [23] to visualize the gene networks and predict function of genes that GSEA identified as being enriched in cervical cancer: kinase LYN, mi-RNA133A, 133B and transcription factor OCT1. The rank criterion was an false discovery rate (FDR) < 0.05, and 500 simulations were performed.

Results

EAF2 mRNA expression in various cancers

To address the mRNA expression of EAF2 in various types of cancers, we performed the difference of EAF2 expression between the cancers and their normal tissues using the Oncomine database, GEPIA2 and GENT2 database. In the Oncomine database, the number of significant unique analyses showing differences of mRNA expression in cancer tissue compared to the normal tissue was obtained with the parameters of p-value < 0.01, fold-change > 1, and gene ranking in the top 10%. The comparison of expression level between each type of cancer vs. normal counterpart revealed the upregulation of EAF2 in 10 cancers, including breast, cervical, esophageal, head and neck, leukemia and lung cancers, et al (Fig. 1a, Table1). The increase of EAF2 in cervical cancer was greatest. However, EAF2 was down-regulated in 12 tumors (Fig. 1a). We further analyzed the expression of EAF2 between 33 types of human cancer and their normal tissues with the expression data retrieved from combined TCGA and GTEx data using GEPIA2 tools (Fig. 1b). Among the 33 cancer types, 4 displayed significantly higher EAF2 expression levels compared to their normal counterparts and only one cancer displayed lower EAF2 expression levels. In the GENT2 database, analyzed using the U133Plus2 platform, EAF2 expression was upregulated in certain cancer types, including bladder, blood, cervical, and esophageal cancers, et al (Fig. 1c). The data revealed the significantly increased expression of EAF2 in various cancer types. Expression levels of EAF2 in cervical cancer were significantly higher in all three databases compared to their normal tissue.

EAF2 mRNA and protein expression in cervical cancer

To observe the expression of EAF2 in various subtypes of cervical cancer, we analyzed each individual subtype dataset from the Oncomine database (Fig. 2a-c). The expression of EAF2 of high grade cervical squamous intraepithelial neoplasia (HGCSIN), cervical squamous cell carcinoma (CSCC) and cervical cancer were higher than that of cervix squamous epithelium (CSE) and cervix uteri (CU). Increased expression of EAF2 in cervical cancer was also confirmed in the TCGA dataset using the UALCAN tool (Fig. 2h). We next sought to verify this trend at the protein level between glandular cells (normal healthy

tissue) and cervical squamous cell carcinoma (tumor tissue). In the immunohistochemistry data from the Human Protein Atlas project, cervical cancer patients' samples had weak intensity, whereas normal cervical squamous cell in healthy cervical showed negative intensity (Fig. 2i,j). Overall, expression data in multiple databases suggested that EAF2 expression could be augmented in cervical cancer tissues compared to normal counterparts.

Association between EAF2 expression and clinical characteristics of cervical cancer patients

We investigated the association between EAF2 mRNA expression and the clinicopathological characteristics of cervical cancer using TCGA data through UALCAN. Compared to the normal tissue, expression of EAF2 was augmented regardless of cancer stage (S1, S2, S3, and S4), race (Caucasian, African-American, and Asian), weight (normal weight, extreme weight, obese, extreme obese), age (21–40, 41–60, 61–80, and 81–100 Years), nodal metastasis statues and histological subtype (Fig. 3a-f, Table 2). Interestingly, EAF2 expression decreased with the increase of individual cancer stage. EAF2 expression in cervical adenosquamous carcinoma (CAC).and cervical squamous cell carcinoma remains lower compared to other tissue types. In addition, EAF2 expression decreased with lymph node metastasis. Promoter methylation is one of the essential epigenetic regulatory factors of gene expression. Promoter methylation was significantly increased in CC tumors compared to normal tissue counterparts in the UALCAN analysis of TCGA data (Fig. 3g, Table3). The level of promoter methylation was also increased regardless of patient characteristics, including cancer stage, tumor grade, tumor histology and nodal metastasis status (Fig. 3h–k, Table 3). However, only the level of methylation of stage2 in the tumor was lower than normal tissue, which was also considered to be the reason for the small number of normal samples. Comparison between EAF2 expression and DNA methylation status suggested that the gene expression might be positively related with some CpG sites. Overall, these data suggested that the expression of EAF2 mRNA increased with the malignant development of CC, and promoter methylation increased in cervical cancer.

Mutations and CNAs of EAF2 gene in cervical cancer

We next analyzed mutations and CNAs of EAF2 in a cohort of cervical cancer patients mainly using cBioPortal web. In the 607 sequenced cervical cancer patients, genetic alteration was found in 32 cervical cancer patients and the mutation rate was 5% (Fig. 4a). TCGA data displayed the most CNAs of EAF2. The CNAs of EAF2 in cervical keratinizing squamous cell carcinoma, cervical non-keratinizing squamous cell carcinoma and cervical squamous cell carcinoma are higher than those of cervix uteri and cervix squamous epithelium in Oncomine database (Fig. 2d-g). Among the CNAs, amplification was the dominant alteration and was found over 5% of the patients (Fig. 4b). CNAs in CC were significantly correlated with the EAF2 expression level in TCGA data (Fig. 4c,d). Specifically, amplification and gain were predominantly correlated with EAF2 expression. These data suggested that the decrease of EAF2 gene expression with the development of cervical cancer may be partly due to CNAs.

Correlation of EAF2 expression and patient survival in cervical cancer

Despite the functional role of EAF2 in human carcinogenesis, the relationship between EAF2 expression and the clinical prognosis of the diseases has not been clarified. Presently, Kaplan–Meier analysis showed the relationship between the expression of EAF2 and survival in cervical cancer patients (Fig. 4e). The prognostic role of EAF2 gene in cervical cancer was also further validated in the GEPIA2 database (Fig. 4f). OS was significantly positively correlated with EAF2 expression in cervical cancer patients.

Analysis of genes co-expressed with EAF2 in cervical cancer

The Function module of LinkedOmics was used to analyze mRNA sequencing data from 273 CESC patients in the TCGA. As shown in the volcano plot (Fig. 5a), 4170 genes (dark red dots) showed significant positive correlations with EAF2, whereas 3331 genes (dark green dots) showed significant negative correlations (FDR < 0.01). The 50 significant gene sets positively and negatively correlated with EAF2 as shown in the heat map (Fig. 5b,c). This result suggests a widespread impact of EAF2 on the transcriptome. The statistical scatter plots for individual genes are shown (Fig. 5d–i). EAF2 expression showed a strong positive association with expression of IQCB1 (Pearson correlation = 0.5284, p = 4.902e–21), ILDR1 (Pearson correlation = 0.5267, p = 6.935e–21), and ASTE1 (Pearson correlation = 0.4782, p = 5.245e–17), while negative association with expression of MMS19 (Pearson correlation = 0.43, p = 1.029e–13), NAT8L (Pearson correlation = 0.4116, p = 1.384e–12), and WWC2 (Pearson correlation = 0.4079, p = 2.273e–12). Our findings suggested that expression of EAF2 and these related genes might be closely correlated and may contribute to a signaling pathway in CC.

GO and KEGG pathway analyses of co-expression genes correlated with EAF2 in cervical cancer

Lastly, GO (CC, BP and MF) term and KEGG pathway were analyzed by GSEA in the Link-Interpreter module of LinkedOmics. GO term analysis showed that genes differentially expressed in correlation with EAF2 were located mainly in thenucleoid preribosome and polysome, where they participate adaptive immune response, interleukin-4 production and hippo signaling. They act as Ran GTPase binding, oxidoreductase activity, acting on CH-OH group of donors and helicase activity (Fig. 6a-c, Additional files 1-3: Tables S1-S3). KEGG pathway analysis showed enrichment in the primary immunodeficiency, hematopoietic cell lineage, intestinal immune network for IgA production, staphylococcus aureus infection and chemical carcinogenesis (Fig. 6d, Additional file 4: Table S4).

EAF2 networks of kinase, miRNA or transcription factor targets in cervical cancer

To further explore the targets of EAF2 in cervical cancer, we analyzed the kinase, miRNA and transcription factor target networks of significantly correlated gene sets generated by GSEA (Table 4). The top 3 most significant target networks were the kinase-target networks related primarily to the LYN, SYK and FGR (Additional file 8: Figure S1, Additional file 5: Table S5). The miRNA-target network was associated with (GGGACCA) MIR-133A, MIR-133B, (ATGAAGG) MIR-205 and (CCAGGTT) MIR-490 (Additional file 9: Figure S2, Additional file 6: Table S6). The transcription factor-target network was related mainly to the OCT1_Q5_01 and OCT_Q6 (Additional file 10: Figure S3, Additional file 7: Table S7). The protein-protein

interaction (PPI) network constructed by GeneMANIA revealed correlation among genes for the kinases LYN, miRNA-133A, miRNA-133B and TF OCT1_Q5_01.

Discussion

CC remains one of the major causes of cancer-related death among woman worldwide [1]. For patients with early cervical cancers, surgery or radiotherapy or a combination of both is recommended for the best cure rate [24,25]. However, in most cases, the majority of patients with cervical cancer present with an advanced stage of disease, with limited access to adequate treatment. As a result, the mortality rates are still high [24]. Nowadays, an increasing number of studies have demonstrated the importance of a new type of regulation by epigenetics in specific genes in the progression of cervical cancer [26]. This provides new ideas for the treatment of cervical cancer. The present findings demonstrate that the augmented expression of EAF2 was positively correlated with survival of CC patients, indicating that EAF2 could be a therapeutic target for CC.

Cervical cancer is highly heterogenous genotypically and phenotypically. For example, the most prevalent histological subtypes are squamous cell carcinoma and adenocarcinoma [27]. Several retrospective studies showed that adenocarcinoma are more aggressive than squamous cell carcinoma which can indicate the type of adenocarcinoma patients has a higher risk of developing metastases, resulting in a poorer prognosis [27-30]. To improve the accountability and clinical application of the EAF2 phenotype, additional datasets might be considered into the analysis, reflecting the heterogeneity feature of CC.

As for mechanism of EAF2 in inhibiting tumor development, in Chen study, EAF2 suppresses hypoxia-induced factor 1α transcriptional activity by disrupting its interaction with coactivator CBP/p300 [31]. In Liu study, EAF2 suppresses both TGF-β-induced G1 cell cycle arrest and TGF-β-induced cell migration through a direct interaction with Smad3 [32]. In addition, in prostate cancer, EAF2 regulates DNA repair through Ku70/Ku80 to influence radiation sensitization by androgen deprivation therapy [33]. In glioblastomas, EAF2-HIF1α signaling axis promotes tumorigenesis and malignant progression to activate glycolysis through EZH2 regulation [12]. Furthermore, EAF2 loss is associated with high-risk patients and poor survival [34].

The present systematic study using bioinformatics analyses of public datasets demonstrates, for the first time, the prognostic value of EAF2 in CC. Analyses of the TCGA datasets revealed that the expression of EAF2 decreased with the development of cervical cancer and significant upregulation of EAF2 is positively correlated with the OS of CC patients. Our data also established the relationship between translational relevance and EAF2 mRNA expression in CC patients. Promoter methylation was analyzed from TCGA data through UALCAN web, indicating significantly increased methylation. Among the normal tissue data in TCGA cervical cancer dataset, only three samples have methylation data. In these analyses, only the level of methylation of stage2 in the tumor was lower than normal tissue, which was also considered to be the reason for the small number of normal samples. Although three normal samples were too small for comparison with tumor samples, the difference in methylation was statistically

significant (p = 8.928E-01). To make clear biological importance, additional data might be used for normal control or independently proved by different methods. Thus, these results imply that increased expression of EAF2 in CC, in part, could be caused by one or a combination of these factors. Further studies are needed to more comprehensively explore the detailed molecular mechanisms of this altered biomarker in the progression and prognosis in CC patients.

CNAs can have major genomic implications, disrupting genes and altering genetic content, leading to phenotypic differences [35]. Our study found that the copy number of EAF2 was increased in CC, and that the major type of EAF2 alteration was amplification. Further analysis of CC datasets from TCGA revealed a mutation rate of 5%, more than 5% amplification in CNAs. We speculate that altered EAF2 expression and EAF2 dysfunction in CC may result from alterations in chromosomal structure.

To explore EAF2-related altered pathways in CC, genes that were co-altered along with EAF2 were analyzed. Among the positively correlated genes analyzed in the LinkedOmics database, IQCB1 expression was most highly co-altered along with EAF2 expression, followed by ILDR1 and ASTE1, respectively. IQCB1 was found to be associated with poor prognosis in colorectal cancer [36]. Angulin proteins ILDR1 regulate alternative pre-mRNA splicing through binding to splicing factors TRA2A, TRA2B, or SRSF1 [37]. Overlapping ASTE1 and ATP2C1 genes in human genome implicate for SPCA1, for affecting cytosolic Ca^{2+}-signaling, and in turn perturbing cell division, leading to cell death or to neoplastic transformation [38]. Based on these analyses, we hypothesized that EAF2 might be associated with these positively related genes in some pathways in cancer.

In addition, we utilized LinkedOmics web tools to determine pathways associated with commonly

correlated genes of EAF2 in CC. Moreover, from a functional classification viewpoint, EAF2 and co-altered genes were subjected in GO enrichment analysis of cellular components, biological processes and molecular functions and KEGG enrichment analysis. The results showed that AICDA gene appeared more frequently in the cervical cancer pathway. Tumorigenesis is closely related to the immune response of the body. AICDA and the immune response of the body have been discussed [39,40]. Changes in cytosine methylation of AICDA are associated with tumor resistance mutation in lung cancer [41]. In diffuse large B-cell lymphoma, increased cytosine methylation heterogeneity is associated with poor clinical outcome [42]. We suspect that the AICDA gene has some significance in cervical cancer, while further study is needed.

Enrichment analysis of target gene sets using GSEA can help reveal important networks of target kinases, miRNAs and transcription factors. We found that EAF2 in CC is associated with a network of kinases including LYN, SYK, and FGR. These kinases are src family kinases [43]. LYN has done some researches on tumor-related mechanisms. For example, Lyn knockdown could significantly inhibit the proliferation, migration and invasiveness through its inhibition of apoptosis and autophagy via the PI3K/Akt pathway in melanoma cell lines [44]. LYN inhibited the proliferation, migration, and invasion of gastric cancer cells by being regulated by mir-122-5p [45]. In fact, in Liu study, overexpression LYN promoted cell proliferation,

migration and invasion in cervical cancer. In terms of mechanism, LYN could also promote cervical cancer cells metastasis through activating IL-6/STAT3 pathway [46]. Therefore, we speculate that EAF2 may have a regulatory mechanism with LYN to regulate the occurrence and development of cervical cancer.

Our study identified several miRNAs that were associated with EAF2 including MIR-133A, MIR-133B, MIR-205, MIR-490. The particular miRNAs in our study have been linked to tumor proliferation, apoptosis, cell cycle, invasion and metastasis [47,49]. Hanahan described the 10 hallmark features of tumors, with “continuous proliferation” at the top [50]. Study has shown that overexpression of miR-133a and miR-133b induced G1 cell cycle arrest and inhibited cell proliferation, migration and invasion in vitro [51]. MiR-205 serves as a prognostic factor and suppresses proliferation and invasion by targeting insulin-like growth factor receptor 1 in human cervical cancer [52]. MIR-490-5p and the remaining 5 miRNAs contribute to the metastatic potential of cervical cancer and may aid in prognosis or molecular therapy [53]. Dysregulation of these miRNAs is consistent with the phenotype of EAF2 expression in CC from our study.

We found that EAF2 in CC is associated with a network of transcription factors including OCT1 and OCT. OCT1 was found to influence the genesis and progression of numerous cancers such as colorectal, gastric and prostate cancer, et al [54-56]. In fact, OCT1 regulated FASL gene transcription by interacting with C/EBPβ to influence the development of cervical cancer [57]. Therefore, we suspect that OCT1 is a target of EAF2 in CC. Further studies should test this hypothesi.

This study uses online tools based on the most popular bioinformatics theories to perform target gene analyses on tumor data from public databases. Compared with traditional chip screening, this method has the advantages of large sample size, low cost, and simplicity. This enables large-scale CC genomics research and subsequent functional studies. At the same time, there were some limitations in our study. First, all the data analyzed in our study was retrieved from the online databases, further studies consist of larger clinical sample sizes are required to validate our findings and to explore the clinical application of the EAF2 member in the treatment of CC. Second, we did not assess the potential diagnostic and therapeutic roles of EAF2 in CC, so future studies are needed to explore whether EAF2 could be exploited as diagnostic markers or as therapeutic targets.

Conclusion

In this mining study, we used several online bioinformatic platforms and web tools to systematically analyze the expression, methylation status, mutations and CNAs, correlated genes, and prognostic value of EAF2 in human cervical cancer. The multiomics analysis revealed that the expression of EAF2 decreased with the development of cervical cancer and significant upregulation of EAF2 is positively correlated with the OS of CC patients. The elevated expression of EAF2 could be regulated through promoter methylation and CNAs. The present findings also reveal the importance of EAF2 expression and

possible EAF2-related pathways in cancer progression. The findings indicate the potential of EAF2 as a therapeutic target for CC.

Tables

Table 1. Significant changes of EAF2 expression in transcription level between cervical cancer and normal tissue (ONCOMINE).

Types of cervical cancer vs. normal	Fold Change	P value	t-test	Ref
ɣical Keratinizing Squamous Cell Carcinoma vs. Normal	1.388	0.007	4.157	TCGA Cervix Statistics
ɛrvical Non-Keratinizing Squamous Cell Carcinoma vs. Normal	1.348	5.27E-5	5.660	TCGA Cervix Statistics
ɛrvical Squamous Cell Carcinoma vs. Normal	1.207	7.82E-16	8.831	TCGA Cervix Statistics
Grade Cervical Squamous Intraepithelial Neoplasia Epithelia vs. Normal	1.829	5.01E-4	4.093	Zhai Cervix Statistics
ɹcal Squamous Cell Carcinoma vs. Normal	1.110	6.21E-9	6.602	Scotto Cervix Statistics
ɹcal Squamous Cell Carcinoma vs. Normal	2.240	4.02E-5	4.352	Scotto Cervix 2 Statistics
Cervical Cancer vs. Normal	1.975	1.66E-4	3.950	Pyeon Multi-cancer Statistics

Table 2. The relationship between the EAF2 and the clinicopathologic parameters of cervical cancer (TCGA data).

Parameters	Figure	EAF2		
		mRNA expression	of sample (n)	P-value
Sample types	Figure2h			
Normal		↓	3	
Primary tumor		↑	305	5.84E-13
Individual cancer stages	Figure3a			
Normal		↓	3	
Stage 1		↑	161	1.62E-12
Stage 2		↑	69	9.87E-10
Stage 3		↑	46	2.17E-08
Stage 4		↑	22	2.02E-04
Patient's race	Figure3b			
Normal		↓	3	
Caucasian		↑	209	6.12E-14
African-American		↑	30	5.38E-07
Asian		↑	20	2.85E-06
Patient's weight	Figure3c			
Normal		↓	3	
Normal weight		↑	88	1.66E-12
Extreme weight		↑	73	3.26E-12
Obese		↑	74	3.57E-13
Extreme Obese		↑	12	4.46E-03
Patient's age	Figure3d			
Normal		↓	3	
21–40 Yrs.		↑	92	7.88E-13
41–60 Yrs.		↑	152	1.11E-16
61–80 Yrs.		↑	56	6.17E-12
81–100 Yrs.		↑	3	3.54E-01
Nodal Metastasis status	Figure3e			
Normal		↓	3	
N0		↑	133	<1E-12
N1		↑	60	8.40E-12
Histological subtypes	Figure3f			
Normal		↓	3	
Cervical adenosquamous carcinoma		↑	5	1.19E-02
Cervical squamous cell carcinoma		↑	252	1.64E-12
Endocervical adenocarcinoma of the usual type |		↑	27	2.80E-

Endocervical type of adenocarcinoma			08
Endometrioid adenocarcinoma of endocervix	↑	3	2.22E-01
Mucinous adenocarcinoma of endocervical type	↑	17	3.06E-03

Table 3. Methylation expression of EAF2 and clinicopathological parameters of cervical cancer (TCGA data).

Parameters	Figure	EAF2		
		level	of sample (n)	P-value
Sample types	Figure4a			
Normal		↓	3	
Primary tumor		↑	307	8.93E-01
Individual cancer stages	Figure4b			
Normal		↓	3	
Stage 1		↑	163	8.91E-01
Stage 2		↓	70	8.41E-01
Stage 3		↑	46	8.81E-01
Stage 4		↑	21	7.33E-01
Tumor grade	Figure4c			
Normal		↓	3	
Grade 1		↑	18	9.36E-01
Grade 2		↑	163	8.81E-01
Grade 3		↑	120	8.97E-01
Grade 4		↑	1	N/A
Histological subtypes	Figure4d			
Normal		↓	3	
Cervical adenosquamous carcinoma		↑	6	7.61E-01
Cervical squamous cell carcinoma		↑	254	8.41E-01
Endocervical adenocarcinoma of the usual type | Endocervical type of adenocarcinoma		↑	27	8.02E-01
Endometrioid adenocarcinoma of endocervix		↑	3	3.85E-01
Mucinous adenocarcinoma of endocervical type		↑	17	8.67E-01
Nodal Metastasis status	Figure4e			
Normal		↓	3	
N0		↑	153	9.42E-01
N1		↑	60	8.65E-01

Table 4. The Kinase, miRNA and transcription factor-target networks of EAF2 in cervical cancer (LinkedOmics).

Enriched Category	Geneset	LeadingEdgeNum	FDR
Kinase Target	Kinase_LYN	23	2.014e-2
	Kinase_SYK	18	2.446e-2
	Kinase_FGR	8	3.405e-2
miRNA Target	GGGACCA,MIR-133A,MIR-133B	89	0
	ATGAAGG,MIR-205	51	1.704e-2
	CCAGGTT,MIR-490	24	4.316e-2
Transcription Factor Target	V$OCT1_Q5_01	54	5.534e-3
	V$OCT_Q6	48	4.058e-2

Figures

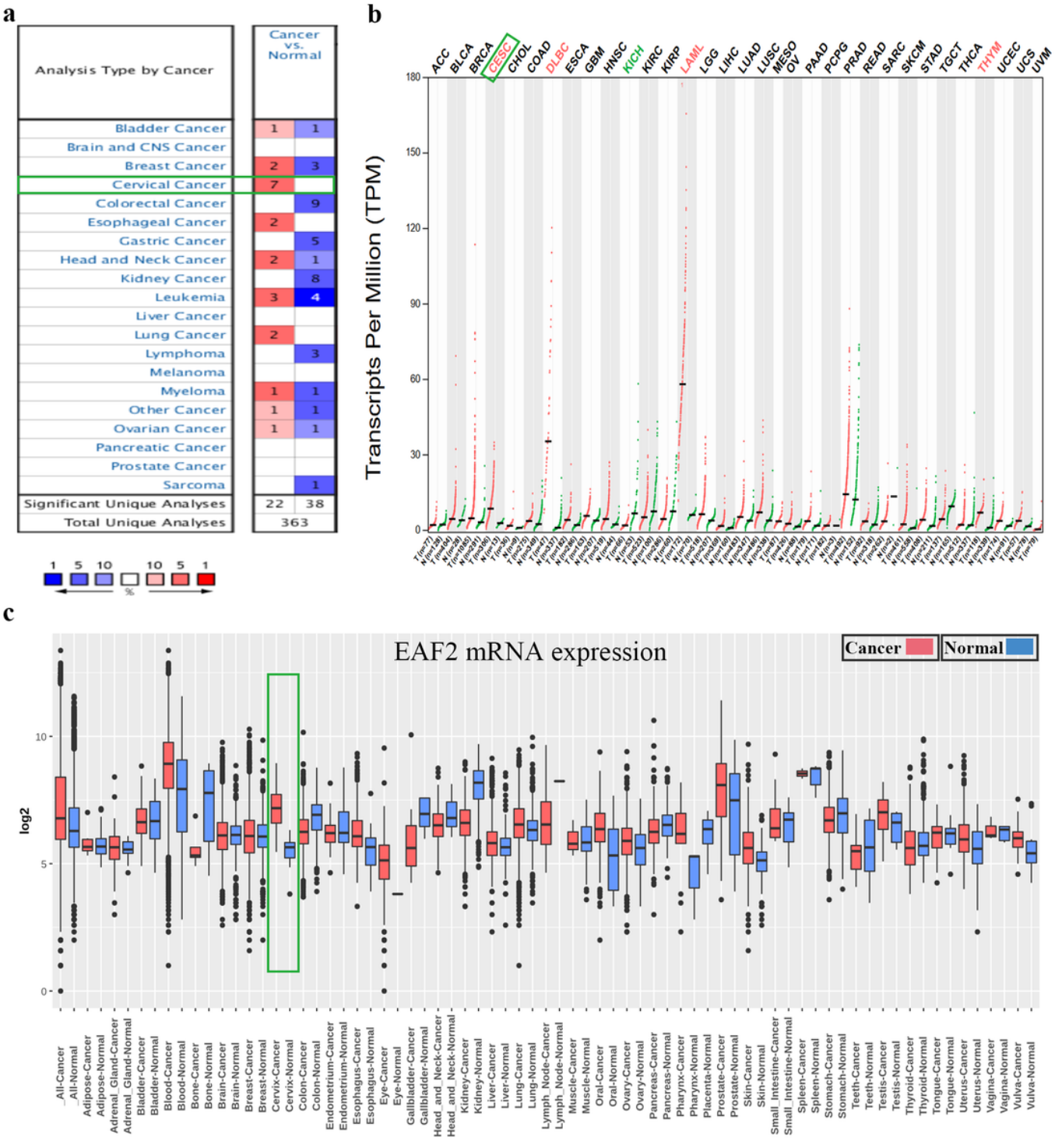

Figure 1

Expression of EAF2 is in various cancer types. a The comparison indicated the number of datasets with EAF2 mRNA overexpression (left column, red) and underexpression (right column, blue) in cancers versus normal tissues. This graphic presentation originated from the Oncomine database (available at https://www.oncomine.org/resource/login.html), and the threshold was designed with the following parameters: p-value of 0.01, fold-change of 1, and gene ranking of 10%. b The expressions of EAF2 in 33 types of human cancer in data from TCGA through GEPIA2 (Gene expression Profiling Interactive

Analysis 2) web (available at https://gepia2.cancer-pku.cn). c Expression pattern of EAF2 mRNA in tumor and corresponding normal tissue: Data concerning EAF2 mRNA expression in various types of cancer were retrieved from the GENT (Gene Expression across Normal and Tumor tissue) database (available at http://medical-genomics.kribb.re.kr/GENT/). Boxes represent the median and the 25th and 75th percentiles. Dots represent outliers. Red boxes represent tumor tissues, and green boxes represent normal tissues.

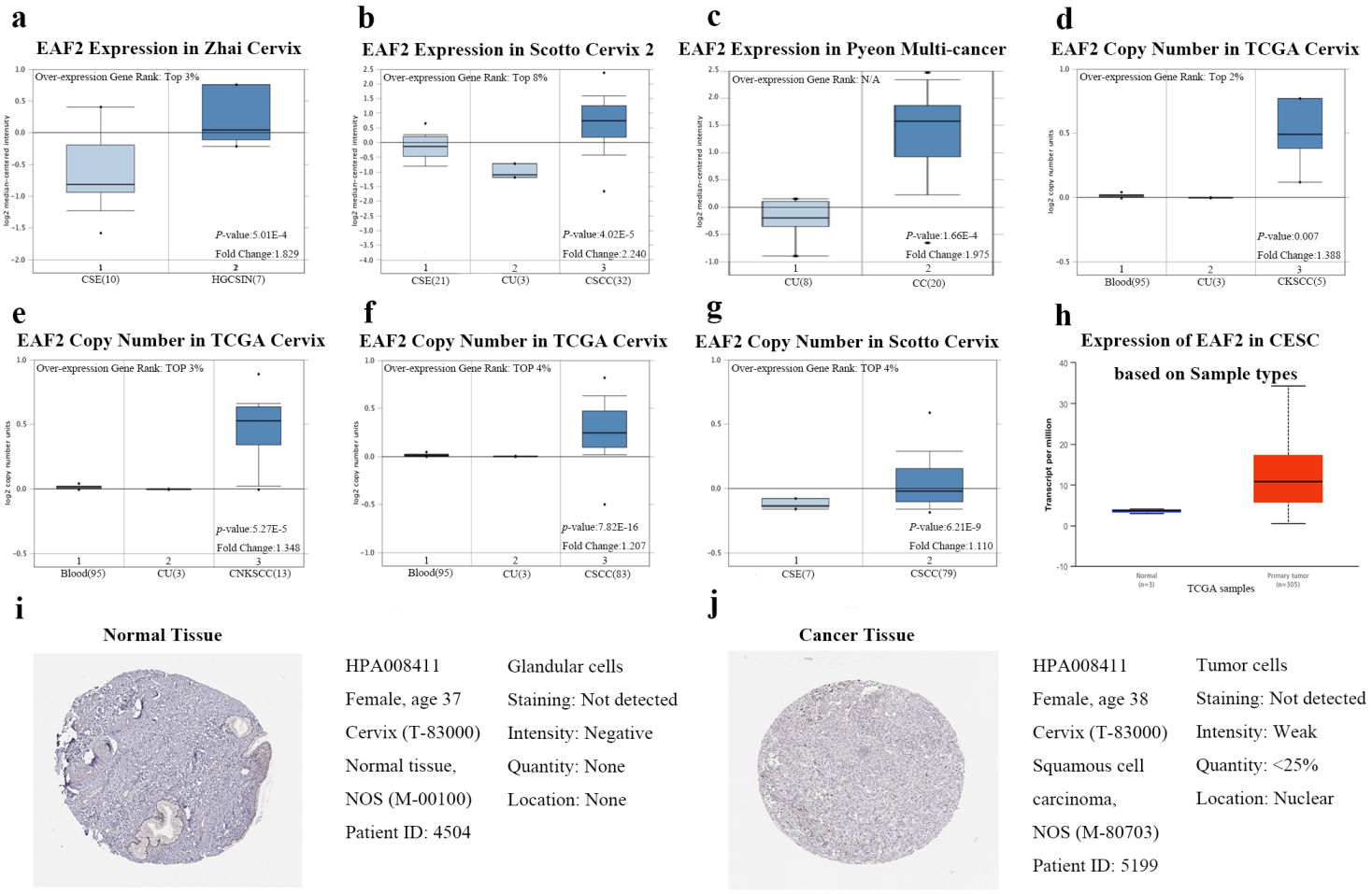

Figure 2

EAF2 mRNA and protein expression are in cervical cancer. Shown are fold change of 1, associated p values of 0.01, and gene ranking of 10%, based on Oncomine database analysis. Box plots show EAF2 mRNA levels, respectively. a Zhai Cervix. b Scotto Cervix. c Pyeon Multi-cancer datasets. d-g Box plots show EAF2 copy number in TCGA Cervix and Scotto Cervix datasets, respectively. h Expression of the EAF2 gene in TCGA database: Box plots showing the EAF2 mRNA expression in CC tumors (red plot) and their normal (blue plot) tissues was derived through UALCAN (http://ualcan.path.uab.edu/index.html). i-j The representative protein expression of EAF2 in CC tissue (squamous cell) and normal tissue (glandular cells) from the immunohistochemistry data were analyzed from the Human Protein Atlas Project (http://www.proteinatlas.org/).

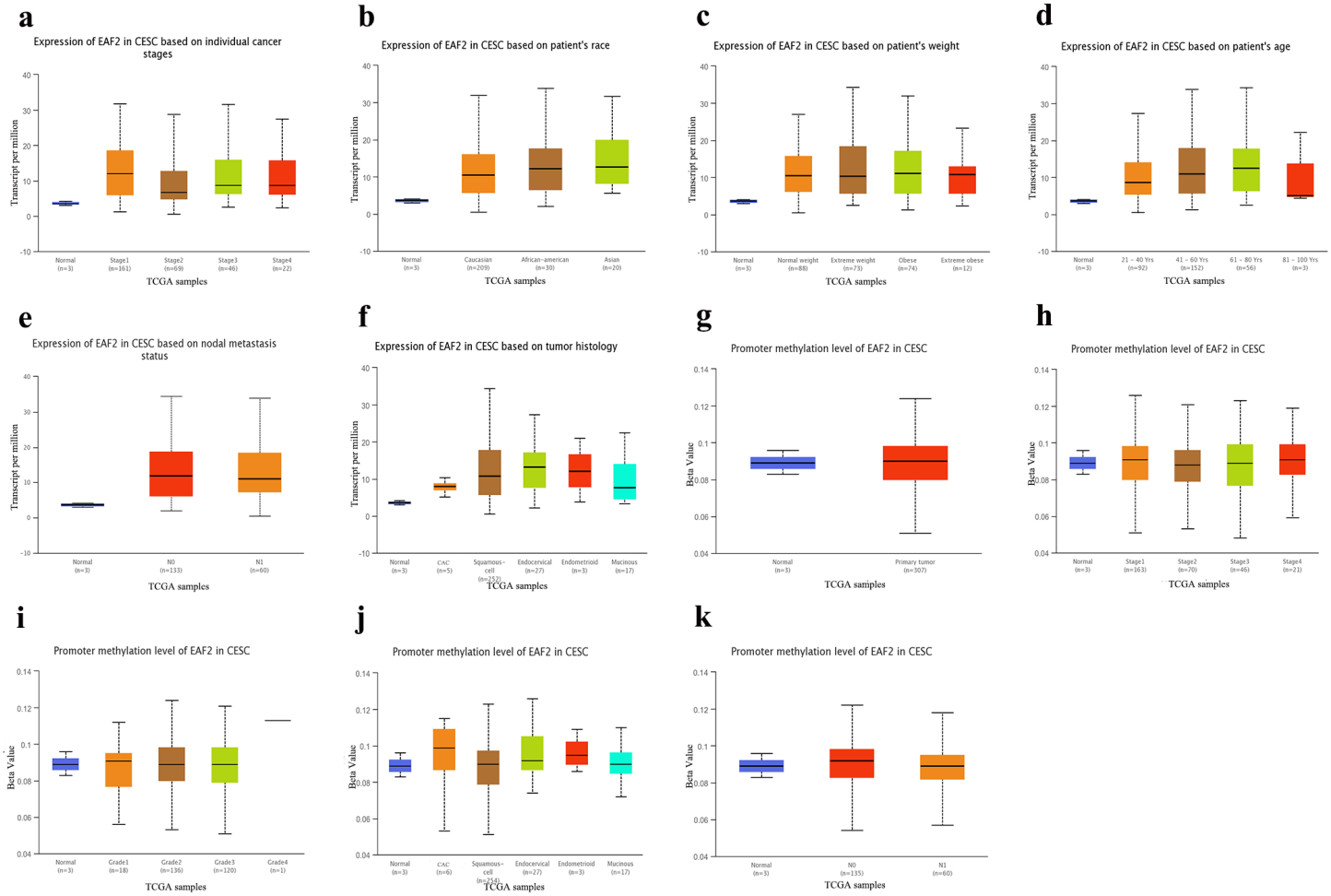

Figure 3

Expression of EAF2 and promoter methylation are shown in cervical cancer using UALCAN web. a-f The EAF2 mRNA expression levels were expressed by box plots for the patient characteristics of individual cancer stages, patient race, patient weight, patient age, nodal metastasis status and histological subtypes, respectively. g-k Promoter methylation of EAF2 in CC tumor (different color plot) and their normal (blue plot) tissues based on normal vs. primary tumor, individual cancer stage, tumor grade, histological subtypes and nodal metastasis status, respectively. The beta value indicates the level of DNA methylation ranging from 0 (unmethylated) to 1 (fully methylated).

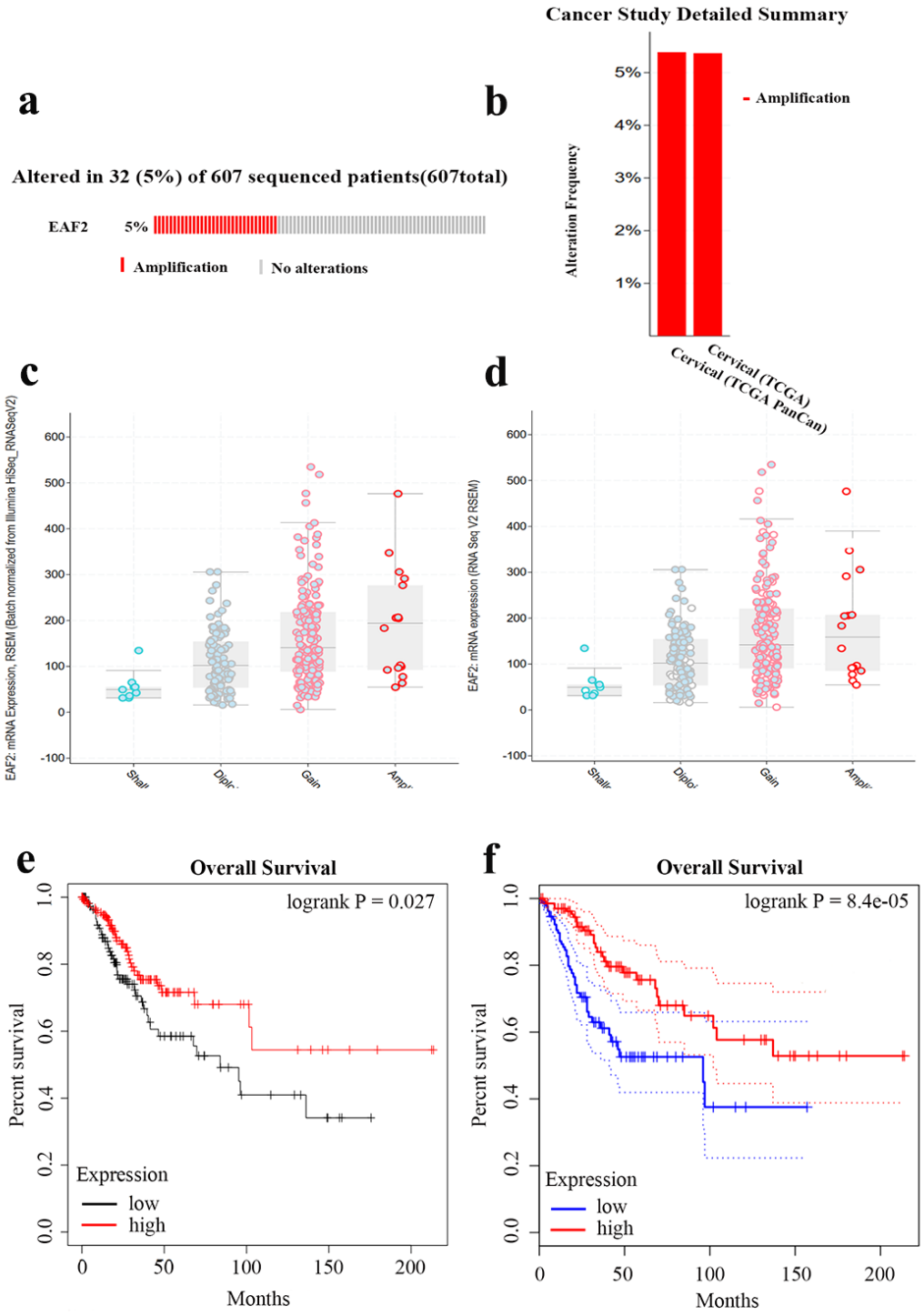

Figure 4

Mutation, CNAs and patient survival of EAF2 expression are shown in cervical cancer. Mutation and CNAs of EAF2 in CC determined using cBioPortal (http://www.cbioportal.org). a Bar chart of mutation rate of EAF2 in cervical cancer. b Frequency of genomic alterations of EAF2 in CC was presented as bar diagram. c-d The graph depicts the correlation between EAF2 expression and CNAs in CC of TCGA data. e-f The relationship between EAF2 expression and OS of cervical cancer patients was in Kaplan–Meier

(http://kmplot.com/analysis/) analysis and GEPIA2 analysis. OS was significantly positively correlated with EAF2 expression in cervical cancer patients.

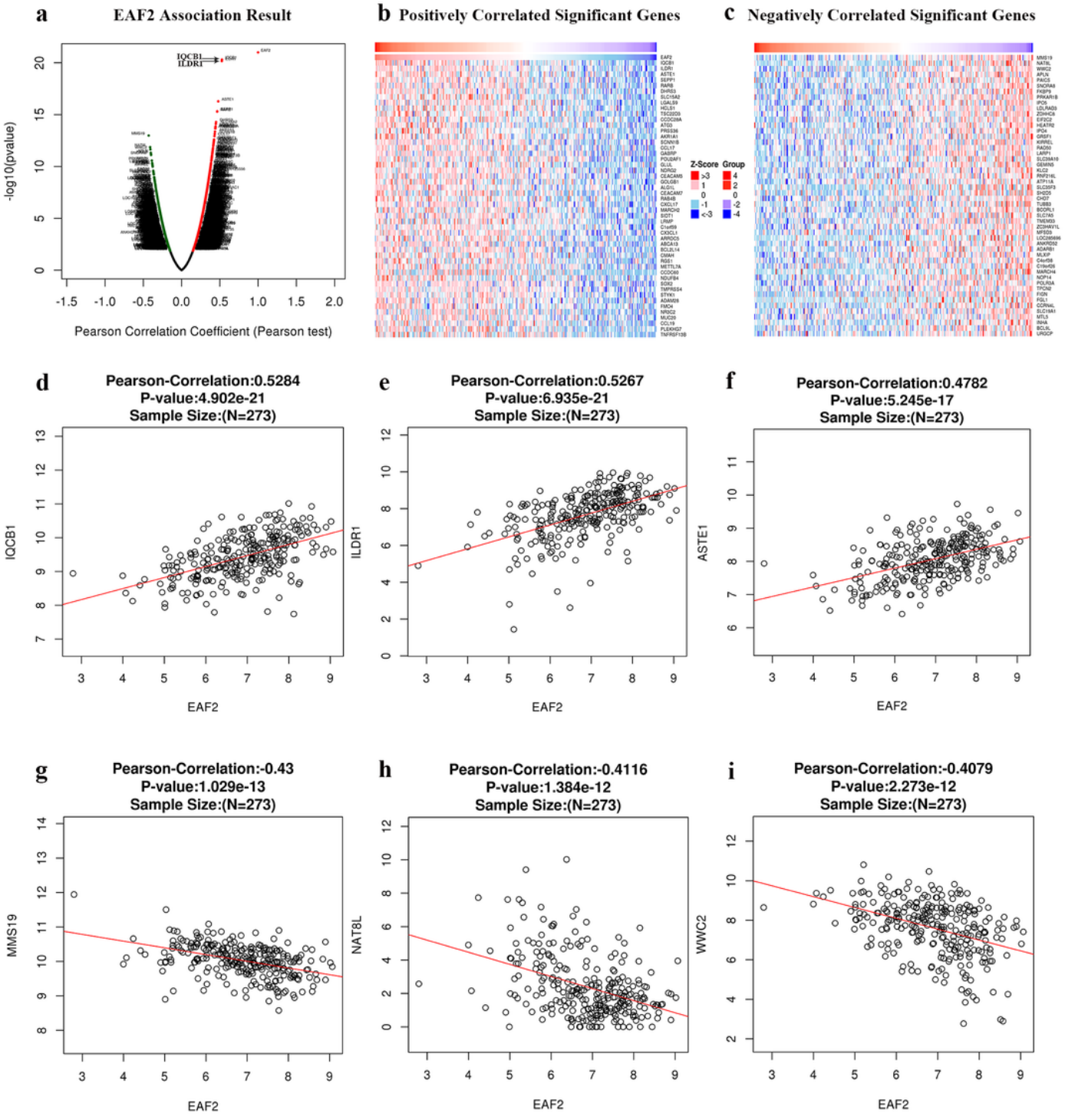

Figure 5

Genes differentially expressed in correlation with EAF2 in cervical cancer (LinkedOmics) (http://www.linkedomics.org/login.php). a A Pearson test was used to analyze correlations between EAF2 and genes differentially expressed in CESC. b-c Heat maps showing genes positively and negatively

correlated with EAF2 in CC (TOP 50), respectively. Red indicates positively correlated genes and green indicates negatively correlated genes. Gene expression correlation analysis for EAF2 (LinkedOmics): d-f the scatter plot shows positively Pearson correlation of EAF2 expression with expression of IQCB1, ILDR1 and ASTE1, respectively. g-i The scatter plot shows negatively Pearson correlation of EAF2 expression with expression of MMS19, NAT8L and WWC2, respectively.

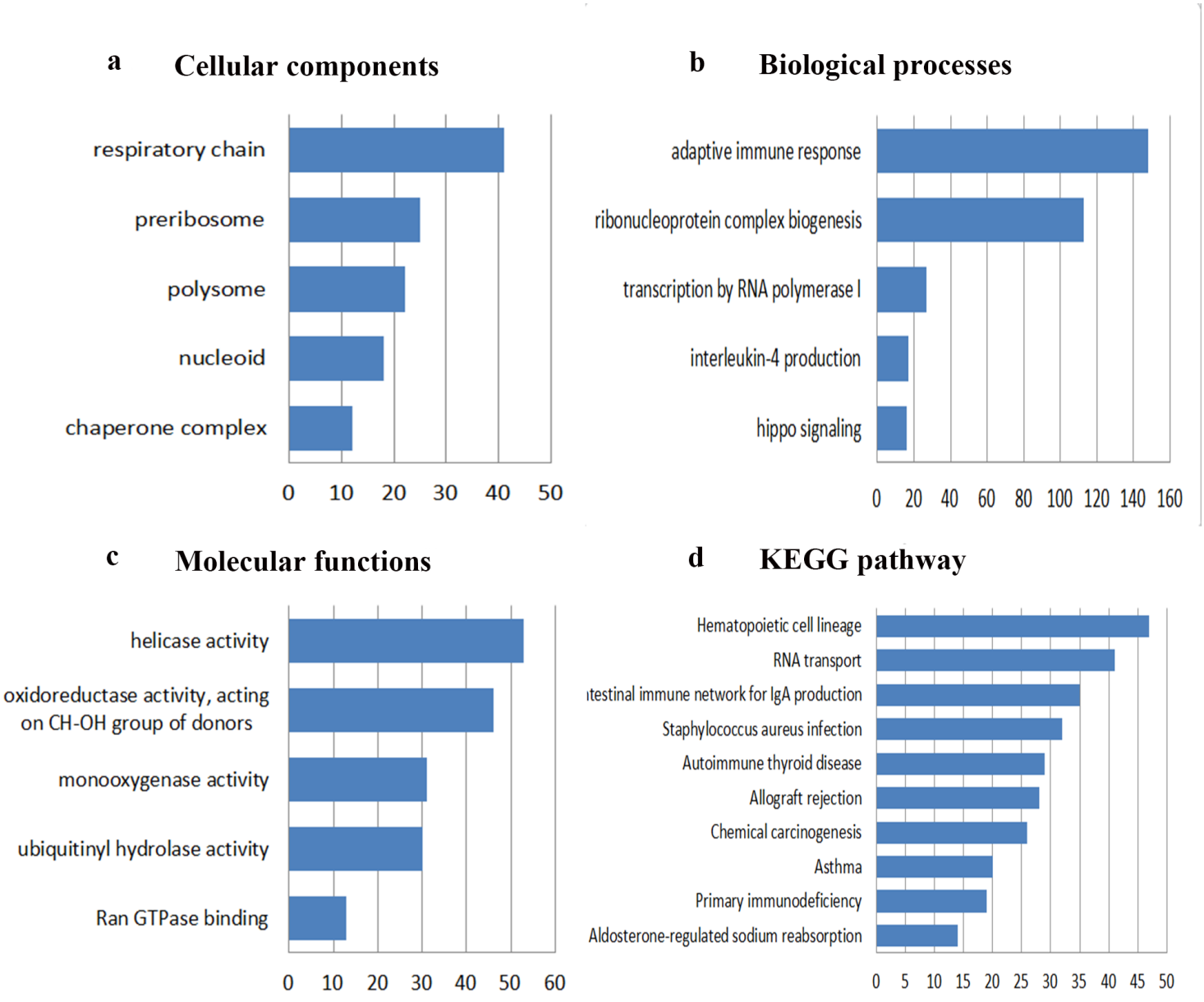

Figure 6

Significantly enriched GO annotations and KEGG pathways of EAF2 in cervical cancer. a Cellular components. b Biological processes. c Molecular functions. d KEGG pathway analysis. The blue column represents the LeadingEdgeNum. The significantly enriched GO annotations and KEGG pathways of EAF2 co-expression genes in CC were analyzed using GSEA.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1TableS1.docx
- Additionalfile2TableS2.docx
- Additionalfile3TableS3.docx
- Additionalfile4TableS4.docx
- Additionalfile5TableS5.docx
- Additionalfile6TableS6.docx
- Additionalfile7TableS7.docx
- Additionalfile8FigureS1.docx
- Additionalfile9FigureS2.docx
- Additionalfile10FigureS3.docx