Study on the regulatory mechanism of EAF2 in the microenvironment of cervical cancer

Fan Guo

Xinjiang Medical University Affiliated Tumor Hospital

Wei na Kong

Xinjiang Medical University Affiliated Tumor Hospital

Gang Zhao

Affiliated Traditional Chinese Medicine Hospital of Xinjiang Medical University

Jie Lv

Xinjiang Medical University Affiliated Tumor Hospital

Jia hui Fan

Xinjiang Medical University Affiliated Tumor Hospital

Yue Wang

Xinjiang Medical University Affiliated Tumor Hospital

Cai ling Ma

Xinjiang Medical University Affiliated First Hospital

Xiu min Ma (✉ maxiumin1210@sohu.com)

State Key Laboratory of Pathogenesis, Prevention and Treatment of High Incidence Diseases in Central Asia. Tumor Hospital Affiliated to Xinjiang Medical University, First Hospital Affiliated to Xinjiang Medical University, Urumqi 830011, China <https://orcid.org/0000-0001-8011-7513>

Research

Keywords: EAF2, cervical cancer, prognosis, immune cells

Posted Date: September 15th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-889206/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Objective

EAF2 plays an important role in transcription elongation and the regulation of gene expression in mammalian cells. EAF2's depletion has been demonstrated to enhance cell proliferation and greatly increase the risk of cancer. Even so, its expression and prognostic role in cervical cancer (CC) remains controversial.

Methods

To solve this issue, we comprehensively investigated the role of EAF2 in CC through various databases including ONCOMINE, UALCAN, Kaplan-Meier Plotter and TIMER.

Results

In all, we found that the EAF2 was highly expressed in CC tissue and was significantly correlated with better patient survival. Among the CNAs, amplification was the dominant alteration. Then the co-expression profile and enrichment analysis of EAF2 were related to the potential signaling pathways. The function of genes such as Kinase LYN, mi-RNA133A-133B and transcription factor OCT1 were also enriched in CC. The positively relation EAF2 expression to 6 immune cells revealed that EAF2 expression may affect development of patients with CC partially due to immune infiltration.

Conclusions

Taken together, our data suggest that EAF2 might be a potential prognostic marker and therapeutic target for CC patients.

Introduction

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]. 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 [3]. There have been improvements in the early detection and treatment of CC, which has improved patient survival. Yet, in most cases, the majority of patients with cervical cancer present with an advanced stage of disease, with a median survival overall of only 18.8 months, limiting access to adequate treatment [4]. 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. 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 [5]. It provides new ideas for the treatment of cervical cancer.

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 [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, colorectal and gastric cancer [10–12]. 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.

In view of the fact that EAF2 expression was elevated in the CC multi-expression dataset, we used numerous publicly expression and patient survival datasets on various online platforms to comprehensively analyze the expression patterns of EAF2 genes in CC patients and their clinical results. In addition, we also analyzed the genes co-altered with EAF2 in CC, and these genes may be related to the mechanisms of EAF2 in the progression and prognosis of CC. Finally, the expression of EAF2 was positively correlated with 6 kinds of immune cells. These data provide supporting evidence for the use of EAF2 as a potential prognostic biomarker and therapeutic target for CC.

Materials And Methods

Transcription-related databases of EAF2 in patients of cervical cancer

Transcriptional expressions of EAF2 between cervical cancer tissues and their adjacent normal tissue counterparts were analyzed using the ONCOMINE database (<https://www.oncomine.org/resource/login.html>) [13], Gene expression Profiling Interactive Analysis 2 (GEPIA2) (<http://gepia.cancer-pku.cn/>) [14], the Gene Expression across Normal and Tumor tissue 2 (GENT2) database (<http://gent2.appex.kr>) [15], and UALCAN web (<http://ualcan.path.uab.edu/index.html>) [16]. In ONCOMINE database, the cut-off of p value and fold change were stated as 0.01 and 1.0, respectively. In all analyses using the remaining other databases, the EAF2 queries were carried out with default Settings to get their respective expression patterns.

EAF2 gene expression in each clinical characteristic

UALCAN web was used to analyze the expression of EAF2 mRNA in each cervical cancer patient in the TCGA datasets. EAF2 mRNA expression in tumor was separately analyzed with patient characteristics of sample types, individual cancer stages, race, age, nodal metastasis status and tumor histology compared to the normal cervical tissue expression by Student's t -test. And $p < 0.05$ was regarded as statically significant.

cBioPortal database

We analyzed the mutations and CNAs of the EAF2 gene using the online open-access cBioPortal website (<http://www.cbioportal.org/>) [17]. The frequency of mutations was estimated from two samples for cervical cancer that can be used in cBioPortal. Use Graph Pad Prism 7.0 to perform relevant statistics on RNA-seq data through the default GISTIC algorithm. The statistical analysis between the two variables used unpaired t test.

Prognostic analysis

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 overall survival (OS). 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 gene was analyzed using the LinkedOmics database (<http://www.linkedomics.org/login.php>) [18]. The LinkFinder module of LinkedOmics was used to study genes differentially expressed in correlation with EAF2 in the TCGA CESC (Cervical squamous cell carcinoma and endocervical adenocarcinoma) 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 GSEA was used to perform analyses of GO (cellular component, biological process and molecular function), KEGG pathways, kinase-target, miRNA-target and transcription factor-target enrichment. The latter two network analyses were based on the Molecular Signatures Database [19]. The Link-Interpreter module of LinkedOmics performs pathway analyses of differentially expressed genes. We used GeneMANIA (<http://www.genemania.org>) 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 [20]. The rank criterion was an FDR < 0.05, and 500 simulations were performed.

TIMER database analysis

TIMER is a comprehensive website for the automatic analysis and visualization of association between immune infiltrate levels and a series of variables (<https://cistrome.shinyapps.io/timer/>) [21]. We assessed the correlation of EAF2 expression with the abundance of six kinds of immune cells (CD8⁺ T cells, macrophage, neutrophils, CD4⁺ T cells, dendritic cell and B cells) in CC via the TIMER algorithm. In

addition, we exploited the correlation module to estimate the correlation of EAF2 with the type markers of above six cells in CC. These gene markers of immune cells were well illustrated in prior studies [22].

Results

EAF2 expression in patients of cervical cancer

In order to study the mRNA expression of EAF2 in different types of cancers, we performed the difference of EAF2 expression between the cancers and their normal tissues using the online databases. In the ONCOMINE database, the comparison of expression level between each type of cancer vs. normal counterpart revealed the upregulation of EAF2 in 10 cancers, while was down-regulated in 12 tumors (Fig. 1a and Table 1). The increase of EAF2 in cervical cancer was greatest. Among the 33 tumors in the GEPIA2 database, EAF2 was highly expressed in 4 types of tumor including cervical cancer, but was low in renal cancer (Fig. 1b). In the GENT2 database, EAF2 expression was up-regulated in certain cancer types, including bladder, blood, and cervical using the U133Plus2 platform analysis (Fig. 1c). The data revealed the expression of EAF2 in various cancer types. Especially, expression level of EAF2 in cervical cancer was significantly higher in all three databases compared to their normal tissue. Moreover, we explored each individual subtype dataset and DNA copy number from the ONCOMINE database. 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) (Fig. 2a-c). CSCC and Cervical non-keratinizing squamous cell carcinoma (CNKSCC) have higher copy numbers than CSE and CU (Fig. 2d-f).

We next analyzed the relationship between mRNA expression of EAF2 and clinicopathological parameters of CC patients with UALCAN, including patients' individual cancer stages, race, age, nodal metastasis status, tumor histology. Compared to the normal tissue, expression of EAF2 was augmented (Fig. 3). The expression of EAF2 decreased with lymph node metastasis and the increase of individual cancer stage. In addition, EAF2 expression in CAC and cervical SCC remains lower compared to other tissue types. Overall, EAF2 expression may serve as a potential diagnostic indicator in CC.

Genetic mutations in EAF2 of cervical cancer patients

Next, we evaluated the mutations and CNAs of EAF2 in a cohort of cervical cancer patients primarily via 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 SCC, cervical non-keratinizing SCC and cervical SCC are higher than those of cervix uteri and cervix squamous epithelium in ONCOMINE database. 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 (Fig. 4c and 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, which was also further validated in the GEPIA2 database (Fig. 5). 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. 6a), 4170 genes (dark red dots) showed significant positive correlations with EAF2, whereas 3331 genes (dark green dots) showed significant negative correlations (false discovery rate [FDR] < 0.01). The 50 significant gene sets positively and negatively correlated with EAF2 as shown in the heat map (Fig. 6b and c). This result suggests a widespread impact of EAF2 on the transcriptome. The statistical scatter plots for individual genes are shown in Fig. 6d–i. EAF2 expression showed a strong positive association with expression of IQCB1 (Pearson correlation = 0.5284, $p = 4.902\text{e-}21$), ILDR1 (Pearson correlation = 0.5267, $p = 6.935\text{e-}21$), and ASTE1 (Pearson correlation = 0.4782, $p = 5.245\text{e-}17$), while negative association with expression of MMS19 (Pearson correlation = 0.43, $p = 1.029\text{e-}13$), NAT8L (Pearson correlation = 0.4116, $p = 1.384\text{e-}12$), and WWC2 (Pearson correlation = 0.4079, $p = 2.273\text{e-}12$). Our findings suggested that expression of EAF2 and these related genes might be closely correlated and may contribute to a signalling pathway in CC.

Enrichment analysis of EAF2 functional networks in cervical cancer

Lastly, GO (CC, BP and MF) term and KEGG pathway were analyzed by gene set enrichment analysis (GSEA) in the Link-Interpreter module of LinkedOmics. GO term analysis showed that genes differentially expressed in correlation with EAF2 were located mainly in the nucleoid preribosome and polysome, where they participate adaptive immune response, interleukin-4 production and hippo signalling. They act as Ran GTPase binding, oxidoreductase activity, acting on CH-OH group of donors and helicase activity (Fig. 7a-c). 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. 7d). In addition, the results showed that AICDA gene appeared more frequently of enrichment analysis in the CC pathway.

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 2). The top 3 most significant kinase-target networks were related primarily to the LYN, SYK and FGR (Fig. S1 and Table S1). The miRNA-target network was associated with (GGGACCA) MIR-133A, MIR-133B, (ATGAAGG) MIR-205 and (CCAGGTT) MIR-490 (Fig. S2 and Table S2). The transcription factor-target network was related mainly to the OCT1_Q5_01 and OCT_Q6 (Fig. S3 and Table S3). The protein-protein interaction network

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

Relationships of EAF2 with immune cells

The TIMER database was searched to estimate the correlations of EAF2 mRNA expression with immune cell infiltration. As illustrated in the scatter plots (Fig. 8a), the expression of EAF2 was positively correlated with immune infiltration of CD8⁺ T cells ($r = 0.14$, $P = 2.00\text{e-}02$), macrophage ($r = 0.056$, $P = 3.51\text{e-}01$), neutrophils ($r = 0.16$, $P = 7.80\text{e-}03$), CD4⁺ T cells ($r = 0.146$, $P = 1.53\text{e-}02$), dendritic cell ($r = 0.059$, $P = 3.32\text{e-}01$) and B cells ($r = 0.229$, $P = 1.23\text{e-}04$). In addition, we focused on the correlations of EAF2 expression with the markers of above six immune cells in CC. After adjusting for purity, the results demonstrated that EAF2 expression was remarkably correlated with above six immune markers in CC (Fig. 8b-g).

Discussion

CC remains one of the major causes of cancer-related death among woman worldwide [1]. Even as treatment for cervical cancer has improved, the mortality rate remains high. EAF2 plays an important role in the regulation of gene expression in cells. EAF2's deletion has been revealed to greatly increase the risk of cancer. However, there are few studies on the expression and function of EAF2 in the occurrence and development of CC.

The present systematic study using bioinformatics analyses of public datasets demonstrates, for the first time, the 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 through UALCAN web, indicating significantly increased in tumor samples, with the statistically significant ($p = 8.928\text{E-}01$). Amplification and gain were predominantly correlated with EAF2 expression. Moreover, EAF2 expression showed a strong association with expression of IQCB1, ILDR1, ASTE1, MMS19, NAT8L and WWC2. The function of genes such as Kinase LYN, mi-RNA133A-133B and transcription factor OCT1 were also enriched in CC. Finally, the positively relation EAF2 expression to 6 immune cells revealed that EAF2 expression may affect development of patients with CC partially due to immune infiltration. Thus, these results imply that EAF2 could be regarded as prognostic marker and therapeutic target in CC.

CNVs can have major genomic implications, disrupting genes and altering genetic content, leading to phenotypic differences [23]. 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 [24]. 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 [25]. Based on these analyses, we hypothesized that EAF2 might be associated with these positively related genes in some pathways in cancer.

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 [26]. LYN has a comprehensive study of tumor-related mechanisms. For example, LYN inhibited the proliferation, migration, and invasion of gastric cancer cells by being regulated by mir-122-5p [27]. In term of CC, study had showed overexpression LYN promoted cell proliferation, migration and invasion through activating IL-6/STAT3 pathway [28]. 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 [29–30]. 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 [31]. MiR-205 serves as a prognostic factor and suppresses proliferation and invasion by targeting insulin-like growth factor receptor 1 in human cervical cancer [32]. MIR-490-5p and the remaining 5 miRNAs contribute to the metastatic potential of cervical cancer and may aid in prognosis or molecular therapy [33]. 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, etc [34–36]. In fact, OCT1 regulated FASL gene transcription by interacting with C/EBP β to influence the development of cervical cancer [37]. Therefore, we suspect that OCT1 is a target of EAF2 in CC. Further studies should test this hypothesis.

Increasing evidence suggests that immune cell infiltration could regard as a significant determinant of both response to immunotherapy and clinical outcome by affecting tumor development and progression [38]. In this study, we found a significant correlation between the expression of EAF2 and the infiltration of the six immune cell types, B cells, CD8⁺ T cells, CD4⁺ T cells, macrophages, neutrophils, and dendritic cells. We further analyzed the cell markers of above six cells, and obtained consistent results. Previous studies have shown that the interaction of CD4⁺ T cell and CD8⁺ T cells influence the immune response and prognosis of tumors [39–40]. Macrophages influence tumor development via interacting with tumor cells to induce lymphangiogenesis in CC [41]. These results indicated that EAF2 are not only as

prognostic indicators, but also may reflect immune status and be implicated in the tumor immune microenvironment.

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, analysis on the transcriptional level could reflect some aspects of tumor progression, but not global changes. We will try our best in the follow-up study to investigate the detailed molecular mechanisms of EAF2 in the progression and prognosis in CC patients.

Conclusion

In this mining study, we used several online bioinformatic platforms and web tools to systematically analyze the expression, CNVs, correlated genes, prognosis and immune cells status of EAF2 in human cervical cancer. The multiple 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.

Abbreviations

CC: Cervical cancer; EAF2: ELL-associated factor 2; GEPIA2: Gene expression Profiling Interactive Analysis 2; GENT2: Gene Expression across Normal and Tumor tissue 2; TCGA: The Cancer Genome Atlas; CNAs: Copy Number Alterations; OS: Overall survival; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; GO: Gene Ontology; CC: Cellular component; BP: Biological process; MF: Molecular function; SCC: Cervical squamous cell carcinoma; CSE: Cervix squamous epithelium; HGCSIN: High grade cervical squamous intraepithelial neoplasia; CU: Cervix uteri; C5CC: Cervical squamous cell carcinoma; CKSCC: Cervical keratinizing squamous cell carcinoma; CNKSCC: Cervical non-keratinizing squamous cell carcinoma.

Declarations

Ethics approval and consent to participate

Not applicable. Our data came from publicly available online databases that had been licensed for use. Therefore, the data used in this study was not anonymous before its use.

Consent for publication

Not applicable.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author (Cailing Ma and Xiumin Ma) upon request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by grants from Xinjiang Medical University's clinical medicine peak discipline (33-0104006020801#), the Xinjiang Uygur Autonomous Region Graduate Research and Innovation Project (XJ2020G174) and the special scientific research project of young medical scientific and technological talents of Xinjiang Uygur Autonomous region Health and Family Planning Commission (WJWY-202154).

Authors' contributions

FG and WK contributed equally to this work. FG and WK designed the study. GZ, JL and JF acquired the information. YW and GZ interpreted the data. FG and WK drafted and revised the manuscript. CM and XM supervised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors wish to thank all the persons who participated in this study, including the reviewers and medics.

References

1. Roth GA, Abate D, Abate KH, et al. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*. 2018;392(10159):1736-1788.
2. Naga CHP, Gurram L, Chopra S, Mahantshetty U. The management of locally advanced cervical cancer. *Curr Opin Oncol*. 2018;30(5):323-329.
3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394-424.

4. Orbegoso C, Murali K, Banerjee S. The current status of immunotherapy for cervical cancer, Reports Pract Oncol Radiother. 2018;23(6):580-588.
5. Zhu H, Zhu H, Tian M, Wang D, He J, Xu T. DNA Methylation and Hydroxymethylation in Cervical Cancer: Diagnosis, Prognosis and Treatment. Front Genet. 2020;11:1-12.
6. Zhong M, Pascal LE, Cheng E, et al. Concurrent EAF2 and ELL2 loss phenocopies individual EAF2 or ELL2 loss in prostate cancer cells and murine prostate. Am J Clin Exp Urol. 2018;6(6):234-244.
7. Kong SE, Banks CAS, Shilatifard A, Conaway JW, Conaway RC. ELL-associated factors 1 and 2 are positive regulators of RNA polymerase II elongation factor ELL. Proc Natl Acad Sci U S A. 2005;102(29):10094-10098.
8. Ai J, Pascal LE, O'Malley KJ, et al. Concomitant loss of EAF2/U19 and Pten synergistically promotes prostate carcinogenesis in the mouse model. Oncogene. 2014;33(18):2286-2294.
9. Xiao W, Zhang Q, Habermacher G, et al. U19/Eaf2 knockout causes lung adenocarcinoma, B-cell lymphoma, hepatocellular carcinoma and prostatic intraepithelial neoplasia. Oncogene. 2008;27(11):1536-1544.
10. Xiao W, Zhang Q, Jiang F, Pins M, Kozlowski JM, Wang Z. Suppression of prostate tumor growth by U19, a novel testosterone-regulated apoptosis inducer. Cancer Res. 2003;63(15):4698-4704.
11. Wang Z, Qiao Z, Wang D, Hahn J, Ai J. Pirin down-regulates the EAF2/U19 protein and alleviates its growth inhibition in prostate cancer cells. Prostate. 2014;74(2):113-120.
12. Jo YS, Kim SS, Kim MS, Yoo NJ, Lee SH. Candidate Tumor Suppressor Gene EAF2 is Mutated in Colorectal and Gastric Cancers. Pathol Oncol Res. 2019;25(2):823-824.
13. Rhodes DR, Yu J, Shanker K, et al. ONCOMINE: A Cancer Microarray Database and Integrated Data-Mining Platform. Neoplasia. 2004;6(1):1-6.
14. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: A web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017;45(W1):W98-W102.
15. Park SJ, Yoon BH, Kim SK, Kim SY. GENT2: An updated gene expression database for normal and tumor tissues. BMC Med Genomics. 2019;12(S5):1-8.
16. Chandrashekar DS, Bashel B, Balasubramanya SAH, et al. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia (United States). 2017;19(8):649-658.
17. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013;6(269):PI1-PI1.

18. Vasaikar SV, Straub P, Wang J, Zhang B. LinkedOmics: Analyzing multi-omics data within and across 32 cancer types. *Nucleic Acids Res.* 2018;46(D1):D956-D963.
19. Liberzon A, Subramanian A, Pinchback R, Thorvaldsdóttir H, Tamayo P, Mesirov JP. Molecular signatures database (MSigDB) 3.0. *Bioinformatics.* 2011;27(12):394-424.
20. David WF, Donaldson SL, Ovi C, et al. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res.* 2010;38(suppl-2):W214-W220.
21. Li T, Fan J, Wang B, et al. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. *Cancer Res.* 2017;77(21):e108–e110.
22. Danaher P, Warren S, Dennis L, et al. Gene expression markers of Tumor Infiltrating Leukocytes. *J Immunother Cancer.* 2017;5(1):1-15.
23. Urrutia E, Chen H, Zhou Z, Zhang NR, Jiang Y. Integrative pipeline for profiling DNA copy number and inferring tumor phylogeny. *Bioinformatics.* 2018;34(12):2126-2128.
24. Chang Z, Miao X, Zhao W. Identification of Prognostic Dosage-Sensitive Genes in Colorectal Cancer Based on Multi-Omics. *Front Genet.* 2020;10.
25. Micaroni M, Malquori L. Overlapping ATP2C1 and ASTE1 genes in human genome: Implications for SPCA1 expression. *Int J Mol Sci.* 2013;14(1):394-424.
26. Fitzer-Attas CJ, Lowry M, Crowley MT, et al. Fcy receptor-mediated phagocytosis in macrophages lacking the Src family tyrosine kinases Hck, Fgr, and Lyn. *J Exp Med.* 2000;191(4):669-681.
27. Meng L, Chen Z, Jiang Z, et al. MiR-122-5p suppresses the proliferation, migration, and invasion of gastric cancer cells by targeting LYN. *Acta Biochim Biophys Sin.* 2020;52(1):394-424.
28. Liu S, Hao X, Ouyang X, et al. Tyrosine kinase LYN is an oncotarget in human cervical cancer: A quantitative proteomic based study. *Oncotarget.* 2016;7(46):75468-75481.
29. Yue Z, Xue FX, Zhang YS. miR-205 mediates the inhibition of cervical cancer cell proliferation using olmesartan. *J Renin-Angiotensin-Aldosterone Syst.* 2016;17(3):1470320316663327.
30. Wu L, Zhang M, Qi L, Zu X, Wang L. ERα-mediated alterations in circ_0023642 and miR-490-5p signaling suppress bladder cancer invasion. *Cell Death Dis.* 2019;10(9):1-11.
31. Qiu T, Zhou X, Wang J, et al. MiR-145, miR-133a and miR-133b inhibit proliferation, migration, invasion and cell cycle progression via targeting transcription factor Sp1 in gastric cancer. *FEBS Lett.* 2014;588(7):1168-1177.

32. Pang H, Yue X. MiR-205 serves as a prognostic factor and suppresses proliferation and invasion by targeting insulin-like growth factor receptor 1 in human cervical cancer. *Tumour Biol.* 2017;39(6):101042831770130.
33. Ding H, Wu YL, Wang YX, Zhu FF. Characterization of the MicroRNA Expression Profile of Cervical Squamous Cell Carcinoma Metastases. *Asian Pacific J Cancer Prev Apjcp.* 2014;15(4):1675-1679.
34. Wang Y, Song G, Chen J, et al. Elevated OCT1 participates in colon tumorigenesis and independently predicts poor prognoses of colorectal cancer patients. *Tumor Biol.* 2016;37(3):3247-3255.
35. Qian J, Kong X, Deng N, et al. OCT1 is a determinant of synbindin-related ERK signalling with independent prognostic significance in gastric cancer. *Gut.* 2015;64(1):37-48.
36. Obinata D, Takayama KI, Urano T, et al. Oct1 regulates cell growth of LNCaP cells and is a prognostic factor for prostate cancer. *Int J Cancer.* 2012;130(5):1021-1028.
37. Wu S, Wang S, Fu Y, et al. A novel mechanism of rs763110 polymorphism contributing to cervical cancer risk by affecting the binding affinity of C/EBP β and OCT1 complex to chromatin. *Int J Cancer.* 2017;140(4):756-763.
38. Bindea G, Mlecnik B, Tosolini M, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity.* 2013;39(4):782–795.
39. Aoki H, Ueha S, Shichino S, et al. TCR repertoire analysis reveals mobilization of novel CD8+T cell clones into the cancer-immunity cycle following anti-CD4 antibody administration. *Front. Immunol.* 2019;10(1):1–13.
40. Knocke S, Fleischmann-Mundt B, Saborowski M, et al. Tailored Tumor Immunogenicity Reveals Regulation of CD4 and CD8 T Cell Responses against Cancer. *Cell Rep.* 2016;17(9):2234–2246.
41. Ding H, Cai J, Mao M, et al. Tumor-associated macrophages induce lymphangiogenesis in cervical cancer via interaction with tumor cells. *Apmis.* 2014;122(11):1059–1069.

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
Cervical Keratinizing Squamous Cell Carcinoma vs. Normal	1.388	0.007	4.157
Cervical Non-Keratinizing Squamous Cell Carcinoma vs. Normal	1.348	5.27e-5	5.660
Cervical Squamous Cell Carcinoma vs. Normal	1.207	7.82e-16	8.831
High Grade Cervical Squamous Intraepithelial Neoplasia Epithelia vs. Normal	1.829	5.01e-4	4.093
Cervical Squamous Cell Carcinoma vs. Normal	1.110	6.21e-9	6.602
Cervical Squamous Cell Carcinoma vs. Normal	2.240	4.02e-5	4.352
Cervical Cancer vs. Normal	1.975	1.66e-4	3.950

Table 2 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

EAF2 mRNA expression 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. b The expressions of EAF2 in 33 types of human cancer in data from TCGA through GEPIA2 web. 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 GENT2. Red boxes represent tumor tissues, and blue boxes represent normal tissues.

Figure 2

EAF2 mRNA and protein expression in cervical cancer. a-c Box plots show EAF2 mRNA levels in, respectively, the Zhai Cervix, Scotto Cervix, and Pyeon Multi-cancer datasets. d-f Box plots show EAF2 copy number in TCGA Cervix and Scotto Cervix datasets, respectively.

Figure 3

Association between EAF2 expression and clinical characteristics of CC patients. a Sample types. b Individual cancer stages. c patients' race. d patients' age. e Nodal metastasis status. f Tumor histology. Abbreviation: CAC, Cervical Adenosquamous Carcinoma.

Figure 4

Mutation and copy number alterations in EAF2 in CC determined using cBioPortal. a Bar chart of mutation rate of EAF2 in cervical cancer. b Frequency of genomic alterations in EAF2 in CC was presented as bar diagram. c-d The graph depicts the correlation between EAF2 expression and copy number alterations in CC of TCGA data.

Figure 5

Correlation of EAF2 expression and patient survival in CC. a Kaplan–Meier analysis. b GEPIA analysis.

Figure 6

Genes differentially expressed in correlation with EAF2 in cervical cancer (LinkedOmics). 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). Red indicates positively correlated genes and blue indicates negatively correlated genes. d-f The scatter plot shows positively Pearson correlation of EAF2 expression. g-i The scatter plot shows negatively Pearson correlation of EAF2 expression.

Figure 7

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

Figure 8

Relationships of EAF2 with immune cells. a The correlations of EAF2 mRNA expression with immune cell infiltration and a panel of gene markers of immune cells. b CD8+ T cells. c macrophage. d neutrophils. e CD4+ T cells. f dendritic cell. g B cells.

Supplementary Files

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

- [FigureS1.tif](#)
- [FigureS2.tif](#)
- [FigureS3.tif](#)
- [TableS13.docx](#)