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ARTICLE

The Dual Role of ZEB2 in COAD Metastasis and Immunology

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Abstract

Objective: The zinc finger E-box binding homeobox (ZEB2), which can accelerate the nuclear DNA replication by inducing the activation of upstream transcription promoters, was widely considered as an oncogene. Recent study has found that the overexpression of ZEB2 is associated with a better prognosis in hepatocellular carcinoma. However, its roles in tumor growth, metastasis, and immunology are yet to be elucidated in COAD. **Methods:** We used the cBioPortal webtool to analyze and visualize the ZEB2 pan-cancer genomic alteration rate. The module analysis of PPI interaction network was performed using the MCODE tool of Cytoscape software, and the characteristic molecules were selected by cytohHubba tool. CIBERSORTx database was used to analyze the ZEB2 expression in the presence of 22 types of immune infiltrating cells. **Results:** This study found that ZEB2 was aberrantly expressed in most cancer types, and it was significantly downregulated in COAD compared with normal tissue. In addition, our findings also show that overexpression of ZEB2 was associated with a better prognosis in COAD. Mechanistic analysis revealed that overexpression of ZEB2 was associated with the neutrophil extracellular trap formation in COAD. And the results show that ZEB2 expression was significantly correlated with several kinds of immune cell infiltration. **Conclusion:** This study demonstrates that overexpression of ZEB2 was associated with better prognoses in patients with COAD. ZEB2 has close relationship with ACTB, which was highly related to NETs. These findings suggest a dual role of ZEB2 in COAD growth, metastasis, and immunology.

Keywords: ZEB2;colon adenocarcinoma; neutrophil extracellular traps; tumor microenvironment;prognostic biomarke

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Introduction

Colon adenocarcinoma (COAD) is one of the most common malignant tumors in digestive system. Its morbidity and mortality rate ranked the 3rd and 5th in the world respectively, with an increasing trend year by year[1,2]. The five-year survival rate after surgery for early-stage COAD is close to 90%[3]. However, if there is lymph node metastasis or distant metastasis, the five-year survival rate will be markedly reduced, and approximately 25% of COAD patients died from the liver or lung metastasis[4,5]. The high mortality rate of COAD is mainly due to the lack of early symptoms and the lack of ability of clinically used tumor markers to diagnose early COAD. Therefore, investigation of the underlying molecular mechanism of tumor metastasis is crucial to address the unmet clinical need.

Zinc finger E-box binding homeobox (ZEB)2, also known as SMAD-interacting protein-1 (SIP1), contains two zinc finger structural clusters, which can regulate gene transcription by binding with DNA[6]. Previously, ZEB2 was widely considered as an oncogene, can accelerate the nuclear DNA replication by inducing the activation of upstream transcription promoters in cancer cells, and ultimately promote the division of cancer cells[2]. ZEB2 was reported to drive epithelial to mesenchymal transition (EMT) through repression of epithelial genes[7]. Its abnormal expression promotes the malignant development of tumors, plays an important role in drug resistance, tumor stem cell-like properties, apoptosis, survival, cell cycle arrest, tumor recurrence, metastasis, etc[1,8,9]. However, we also noticed that there were an increasing number of studies have reported its overexpression is associated with a better prognosis in some kinds of tumor. Cai Muyan et al. have shown that overexpression of ZEB2 in peritumoral liver tissue correlated with favorable prognostic of patients with hepatocellular carcinoma[10]. Interestingly, Louise Hill et al. postulated a double-negative feedback loop formed by ZEB2 and several microRNA species (predominantly miR-200 family) that controls EMT in cancer cells[11]. On the contrary, Carolina Martinez-Ciarpaglini et al. have reported that ZEB2 was upregulated and miR200 expression was reduced in tumor budding areas in colon cancer[12] . These inconsistent findings caught our attention. And we hypothesized that ZEB2 plays a dual role of promotion and suppression in COAD. Therefore, it is particularly important to elucidate the regulatory functions and molecular mechanisms of ZEB2 to provide new directions and strategies for the clinical treatment of COAD.

Neutrophils are generally considered defensive responses to tumor cells, but recent evidence suggests that tumors regulate neutrophil function to support tumor growth and progression, and are mainly involved in promoting tumorigenesis, metastasis, angiogenesis, and immunosuppression[13]. Studies have shown that neutrophils may exert their functions through chemotaxis, phagocytosis, degranulation and formation of Neutrophil Extracellular Traps (NETs)[14]. NETs are reticular structures containing depolymerized DNA filaments and granular contents excreted by neutrophils under certain stimuli, which induce a new type of cell death distinct from apoptosis and necrosis[15]. NETs are also rich in pro-inflammatory molecules and have been implicated in various sterile inflammatory conditions. Studies have shown that neutrophils can promote distant metastasis by capturing circulating tumor cells through NETs, leading to the spread of tumor cells. Animal experiments have shown that elevated neutrophil levels in colon cancer liver metastasis models are closely related to the formation of NETs and tumor metastasis[16]. Linbin Yang et al. have reported that

DNA of neutrophil extracellular traps promotes cancer metastasis via CCDC25[17]. It has been reported that colon cancer-derived exosomes induce neutrophil recruitment and NETs formation by transferring mutated KRAS to neutrophils, ultimately leading to disease progression[18]. Recent studies have shown that NETs may act as a physical "shelter" in primary tumor: tumor cells recruit neutrophils into the primary tumor to form NETs, which are wrapped on the surface of tumor cells, reducing the recognition of tumor surface antigens by killer immune cells such as CTL and NK cells, and facilitating tumor immune escape[19]. However, the roles and mechanisms of NETs in the COAD microenvironment are still unclear.

By using multiple bioinformatics techniques, this study investigated the association between ZEB2 expression and prognosis in COAD. And identified that the overexpression of ZEB2 is closely related to NETs and immune cell infiltration in COAD.

To conclude, our data provided insight into the underlying molecular mechanism of Colon adenocarcinoma, which might help in improving therapeutic outcome.

Materials and Methods

ZEB2 Expression Pattern in Human Pan-Cancer

Pan-cancer sequencing data from the TCGA-Pancancer cohort, normal human tissue data from the Genotype-tissue expression (GTEx) database and Broad Institute Cancer Cell Line Encyclopedia (CCLE) were downloaded from UCSC Xena (https://xenabrowser.net/). The expression profiles were normalized via transcripts per kilobase million (TPM) conversion, and the log2 (TPM+1) conversion was used for subsequent data process[20]. In addition, in order to further study the difference of ZEB2 expression among 33 tumors, we calculated the logfc value of ZEB2 in each tumor in batches. Raw count mRNA expression profiles of COAD were curated from The Cancer Genome Atlas (TCGA) through the GDC data portal (https://portal.gdc.cancer.gov/). Immunohistochemical (IHC) images were acquired from The Human Protein Atlas database (https://www.proteinatlas.org/)[21].

Genomic Alterations Analysis of Human in Human Pan-Cancer

The cBioPortal (http://www.cbioportal.org) is an international public repository that stores massive cancer genomics datasets, and is widely used to identify the molecular data in cancer tissues or understand the associated genetics, epigenetics, gene expression, and proteome[22,23]. To investigate the genomic alteration (such as mutation, amplification, deep deletion and multiple alterations) rates of ZEB2 in human pan-cancer, we used the cBioPortal webtool to analyze and visualize the rate into bar plots.

GEO Expression Datasets

To explore the expression level of ZEB2 and relevant biological processes in COAD patients, we searched datasets from the GEO (https://www.ncbi.nlm.nih.gov/geo/) database with the keywords " colon adenocarcinoma" [All Fields] AND "Homo sapiens"[porgn] AND "gse"[Filter]. The inclusion and exclusion criteria were as follows: datasets should be the whole-genome expression data of mRNA; expression data should be obtained from COAD patients received no preoperative chemotherapy or radiation therapy. Finally, The

GSE39582 (COAD, T = 566, N = 19) and GSE87211 (COAD, T = 203, N = 160) datasets were collected from the Gene Expression Omnibus GEO database.

Prognostic Analysis

UCSC Xena database was used to download prognostic information of COAD patients, including overall survival (OS) time and progression-free survival (PFS) time. and bivariate ZEB2 expression levels were used to perform Kaplan–Meier curves analysis by the "survminer" R package[24]. Briefly, the samples in the TCGA dataset were classified into high-expression and low-expression groups according to the median value of ZEB2 expression.

KEGG and GO analyses to determine ZEB2-related biological pathways

We used "ClusterProfiler" and "DOSE" R language packages to perform pathway enrichment analysis to investigate the biological pathways that correlate with ZEB2. The samples in the TCGA dataset were classified into high-expression and low-expression groups according to the median value of ZEB2 expression. Then, we analyzed the differentially expressed genes (DEGs) using the "DEseq2" R package with a P-value <0.05 as the threshold. Kyoto Encyclopedia of Genes and Genomes (KEGG) was performed to determine the biological pathways. Gene Ontology (GO) enrichment analysis was performed to determine the biological processes, molecular functions, and cellular components that were altered in a ZEB2-dependent manner in the COAD samples.

PPI interaction network analysis of ZEB2 and NETs

NETs-associated regulatory genes comprising 23 genes that were released by human neutrophils in NETs was identified via protein enrichment in a previous study[25]. Then we put them into the string to get PPI network. The module analysis of PPI interaction network was performed using the MCODE tool of Cytoscape software, and the characteristic molecules were selected by cytoHubba tool[26].

Immune Infiltration Analysis

CIBERSORT algorithm, with the support of the web tool (https://cibersort.stanford.edu/)[27]. CIBERSORT algorithm is a comprehensive resource for systematical analysis of immune infiltrates across diverse cancer types. To explore the relationship between the ZEB2 expression level and immune infiltration in COAD, we used the CIBERSORTx database (https://cibersort.stanford.edu/) to analyze the ZEB2 expression in the presence of 22 types of immune infiltrating cells, including B cells memory, B cells naive, dendritic cells activated, dendritic cells resting, eosinophils, macrophages M0, macrophages M1, macrophages M2, mast cells activated, mast cells resting, monocytes, neutrophils, NK cells activated, NK cells resting, plasma cells, T cells CD4 memory activated, T cells CD4 memory resting, T cells CD4 naïve, T cells CD8, T cells follicular helper, T cells gamma delta, and T cells regulatory (Tregs). In addition, we visualized the statistical Spearman correlations between the mRNA expression level of ZEB2 and above-mentioned immune cell subsets. The positive and

negative correlation criteria were set as follows: Spearman's r >0 with p-value <0.05, or Spearman's r <0 with p-value <0.05.

Subsequently, we analyzed the relationship between ZEB2 expression, cytokines, chemokines and human leukocyte antigen (HLA).

Statistical Analysis

Statistical analysis was performed with the R software version 4.13 (http://www.r-project.org). The differences between groups were analyzed by Student's t or Fisher's exact tests. The prognostic potential of clinical variables was determined using Cox regression and Kaplan-Meier analyses. Two-tailed P < 0.05 was considered statistically significant. The "DEseq2" and "edgeR" packages were used to analyze Htseq-counts for the TCGA dataset and the RSEM (GEO, log2 scaled) and "ggplot2" packages were used for the plot. The "ClusterProfiler" and "DOSE" packages were used for pathway enrichment analysis.

Results

Pan-Cancer Expression Landscape of ZEB2.

In order to investigate the mRNA expression levels of ZEB2 in pan-cancer, we first integrated and analyzed the TCGA and GTEx databases. The results showed that ZEB2 exhibited inconsistent mRNA expression levels in 33 kinds of human common tumors. The ZEB2 expression was significantly elevated in tumor versus normal tissue in the KIRC, LAML, LLGG, PAAD, and SKCM datasets. In comparison, the results demonstrated that ZEB2 expression level was diminished in 17 types of tumor: ACC, BLCA, BRCA, CESC, COAD, KICH, LIHC, LUAD. LUSC, OV, PRAD. READ, TGCT, THCA, THYM, UCEC, and UCS (Figure 1A, C). We also compared the ZEB2 expression using the data directly from the CCLE. Notably, the downregulated ZEB2 mRNA expression was observed consistently in all gastroenteric tumor cell lines: stomach, large intestine, oesophagus, and small intestine (Figure 1B). Consistent with previous studies, these results indicated that the expression of ZEB2 was unbalanced amongst tumors[28].

Pan-Cancer Analysis of Genetic Alteration of ZEB2

The colorectum is a hollow viscera, different from the material one, inflammatory response is the most common reaction in hollow viscera after injury or even changes of physical and chemical conditions. So, we selected COAD for further investigation and hypothesized that ZEB2 plays a novel role in COAD via a synergistic inflammatory tumor-promoting mechanism.

To elucidate whether the abnormal ZEB2 expression in pan-caner was caused by the genetic alterations of ZEB2, we further analyzed the alteration frequency of ZEB2 using the cBioPortal (TCGA, Pan-Cancer Atlas). And the results demonstrated that the highest ZEB2 alteration frequency was approximately 10% in patients with uterine corpus endometrial carcinoma (UCEC) with "mutation" as the primary type (Figure 1D). Interestingly, patients with colorectal adenocarcinoma (COAD) has the sixth alteration frequency of ZEB2 with "mutation" as the primary type and about 1% "deep deletion". Subsequently, we further investigated the ZEB2 mRNA expression in the deep deletion and the diploid group of

COAD and found that the expression level in the diploid group was significantly higher than that in the deep deletion group (Figure 1E). In summary, the genetic alteration frequency of ZEB2 was not high, which may root in the high conservation characteristics of ZEB2.

Additionally, lollipop plots for ZEB2 genes showing identified variants relative to a schematic representation of the gene. Colored boxes represent specific functional domains. Lollipop represents the variant identified; green lollipops stand for missense mutations and grey lollipops stand for silent mutations.

Expression Profiles of ZEB2 mRNA and Protein in COAD

To verify the abnormal mRNA expression of ZEB2 in COAD, we further analyzed its expression level with microarray datasets (GSE87211 and GSE39582) that were obtained from the Gene Expression Omnibus (GEO) database. The results showed that the mRNA expression level of ZEB2 in COAD was significantly decreased in comparison to normal tissues (Figure 2A). These results were further confirmed by analysis results from both TCGA program and the Genotype-Tissue Expression (GTEx) program (Figure 2B). To further study the protein expression of ZEB2 in COAD, we then performed immunohistochemistry (IHC) staining on histological section from patients with COAD. It's worth noting that the expression of ZEB2 was markedly diminished in poorly differentiated adenocarcinoma (Figure 2C-F) with massive granulocyte infiltration, and that was comparatively higher in well differentiated adenocarcinoma.

Overexpression of ZEB2 Is Associated With a Better Prognosis in COAD

Previous studies have suggested that ZEB2 is closely related to the prognosis and progression of COAD12. Therefore, we analyzed the survival data of COAD from the TCGA database. Surprisingly, we found that a higher expression of ZEB2 was related to a better prognosis in these patients as measured by overall survival (OS) and progression-free survival (PFS) (Figure 2G, H). These results showed that overexpression of ZEB2 may be able to indicate a better prognosis of COAD.

Functional Enrichent Analysis of ZEB2 In COAD

To further elucidate the molecular mechanisms of ZEB2 in regulating COAD, we first normalized the expression profile and then analyzed the differentially expressed miRNAs with COAD patients that were obtained from the GEO database (Figure 3A). Next, we performed Gene Otology (GO) analysis (Figure 3B) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis for COAD. The top 10 significant terms of the KEGG analysis included neuroactive ligand-receptor interaction, systemic lupus erythematosus, calcium signaling pathway, alcoholism, taste transduction, cell adhesion molecules, nicotine addiction, nitrogen metabolism, and cAMP signaling pathway, and these pathways were associated with ZEB2 (Figure 3C). Notably, we found that the ZEB2 expression was correlated with neutrophil extracellular trap formation in COAD.

Overexpression of ZEB2 Is Associated with NETs

To identify proteins that had close relationship with ZEB2, we conducted a co-expression analysis of expression data that was obtained from the TCGA database. As shown in Figure 3D, E, 23 NETs genes were closely related to ZEB2, including MNDA, S100A12, ACTN1, LCP1, etc. We created a protein-protein interaction (PPI) network of the ZEB2 co-expression module. Then, we found that ZEB2 regulates tumors by participating in the NETs regulatory pathway through ACTB.

Correlations Between ZEB2 Expression and Immune Cell Infiltration

Because of the close relationship between ZEB2 and NETs, we hypothesized that ZEB2 plays an important role in tumor immune microenvironment. To further investigate this hypothesis, we analyzed the association between the ZEB2 expression and the immune infiltration level in COAD based on the CIBERSORTx database. The results indicated that ZEB2 expression was positively correlated with B cells naive, macrophages M1, macrophages M2, and T cells CD4 naive. Likewise, we found that ZEB2 expression was negatively correlated with activated T cells CD4 memory activated, T cells CD8, and T cells regulatory (Tregs). Notably, ZEB2 expression was shown to be negatively correlated with mast cells activated while positively correlated with mast cells resting, which implies that overexpression of ZEB2 could suppress the activation of mast cells (Figure 4A-C).

Correlations Between ZEB2 Expression and Immunomodulators

To further investigate whether ZEB2 regulates the pattern of cytokines, chemokines and human leukocyte antigen (HLA) that are crucial to shaping tumor immune microenvironment. By analyzing TCGA expression profile, we found that cytokines and chemokines secreted by tumors (such as CCL1, FOXP3, CCR2, etc.), which have been reported to play a key role in recruiting immune-suppressive cells (such as Tregs, MDSC and TAM). We also found that overexpression of ZEB2 was positively correlated with HLA class I histocompatibility antigen (such as HLA-A, HLA-B, HLA-C, etc.) and HLA class II histocompatibility antigen (such as HLA-DMB, HLA-DMA, HLA-DOB, etc.), which have been reported to act an important role in tumor associated antigen presenting. In general, these results indicated a potential mechanism for ZEB2-associated immune activation.

Discussion

In this study, we first analyzed the expression levels of ZEB2 in pan-cancer based on the integrated data from TCGA and GETx databases. And the results indicate that the expression level of ZEB2 was significantly upregulated in 5 caner types and downregulated in 17 kinds of cancer (Fig. 1A, C). It is worth noting that the expression levels of ZEB2 were found to be downregulated in all gastroenteric tumor cell lines (Figure 1B). The genomic alteration analysis results appeared to rule out the association between ZEB2 and genomic alterations in cancers but stopped short of definitively concluding why ZEB2 was differentially expressed between normal tissues and tumor tissues (Fig 1D, E). A study of gut microbiota shows that ZEB2, a regulator of epithelial-mesenchymal transition, promotes the development of spontaneously invasive colorectal cancer in mice in a microbiota-dependent manner, and

removal of the microbiota completely inhibited ZEB2-induced colorectal cancer16. Others also found that ZEB2 overexpression was highly associated with EMT which was implicated in the early stages of metastasis and/or cancer recurrence changes by disrupting the normal balance between differentiation and drug resistance of cancer cells[29,30]. However, there are several studies have reported that ZEB2 expression is essential for differentiation, maturation, and/or function of CD8+ cytotoxic T cells (CTLs) and NK cells, 2 types of immune cells involved in antitumor immune response[31,32]. This data suggests that the dysregulation of ZEB2 may contribute to COAD progression, it also raises a question that why ZEB2 was abnormally expressed in COAD and its role in the molecular mechanisms underlying COAD.

Therefore, we examined the relationship between ZEB2 expression and the clinical prognosis of COAD patients. First, we replicated the expression analysis of ZEB2 based on the integrated data from TCGA and GETx databases, and we also analyzed the ZEB2 expression level in TCGA database or microarray datasets (GSE87211 and GSE39582) separately. Consistent with above-mentioned results, we found that the ZEB2 expression levels were significantly downregulated in tumor tissues (Fig 2 A, B). Subsequently, we divided the TCGA datasets into high ZEB2 expression group (ZEB2_High Expression) and low ZEB2 expression group (ZEB2_Low Expression) according to the expression level of ZEB2, and performed survival analysis (Figure 2 D). As shown in the figures, the overall survival (OS) and progression-free survival (PFS) of the high ZEB2 expression group were significantly better than those of the low ZEB2 expression group, indicating that ZEB2 expression was positively related to a favorable prognosis of COAD patients. Our findings imply that ZEB2 may play an imperative part in predicting the prognosis of COAD patients and is expected to become a prognostic biomarker in COAD.

To illuminate the underlying molecular mechanism of how ZEB2 affects the prognosis of COAD patients, we further performed differential genes expression analysis between the high and low ZEB2 expression groups of COAD patients. We performed principal component analysis (PCA) on the grouping method before doing the variance, and the results showed that COAD patients could be well differentiated into two groups via this grouping method. We selected differentially expressed genes according to the criterion of $P<0.05$, $|\log FC|>1$. Subsequently, we performed pathway enrichment for selected genes. In GO enrichment analysis (Gene Ontology analysis), Biological Process (BP) found membrane potential regulation, Cellular Component (CC) found synaptic membrane structure, and Molecular Function (MF) found cation channel and other cellular components have statistical significance. KEGG pathway enrichment analysis found that pathways such as neutrophil extracellular trap pathway were closely related to ZEB2. Previous study has identified 23 genes that was released from human neutrophils as NETs characteristic by proteins enrichment. Therefore, we put above-mentioned proteins into the string to get PPI network, and the results showed that 23 proteins were found to be closely related to ZEB2, including MPO, LTF, ACTN1, ENO1, ACTB, LYZ, etc. The actin family was shown to play an essential role in enabling the release of nuclear DNA by neutrophils during NET formation[33]. Therefore, we hypothesized that ZEB2 may mediate the function of NETs by binding ACTB. From this point of view, consistent with previous studies, ZEB2 may be able to promote the proliferation, migration and invasion of COAD. Increasing evidences demonstrate that

NETs are central elements of the innate immune response in cancers[34-37], providing a direction for further exploration of the role of ZEB2 in tumors.

The expression of ZEB2 was positively associated with the infiltration of mast cells resting, B cells naive, macrophages M1, macrophages M2, and T cells CD4 naive. The relationships between the ZEB2 expression and immunosuppressive and immunostimulatory genes were also analyzed. We found that ZEB2 overexpression is closely associated with most kinds of cytokines, chemokines, and HLA histocompatibility antigen. The negative correlation between immunosuppressants and immunostimulants in ZEB2 high expression group further reflects the complexity of the tumor microenvironment.

However, we found that overexpression of ZEB2 was positively correlated with HLA class II histocompatibility antigen, which have been reported to act an important role in tumor associated antigen presentation and CD4+ T cell activation[38]. Since CD4+T cells can play an antitumor role by directly inhibiting the cell cycle of tumor cells and indirectly pro-inflammatory/immune effects, we thought that may explain by how the ZEB2 overexpression was associated with better prognosis in patients with COAD[39]. In general, these results indicated a potential mechanism for ZEB2-associated immune activation

Conclusion

In conclusion, our studies provide novel insight into the potential role of ZEB2 in COAD. Our studies demonstrate that ZEB2 may serve as an effective prognostic indicator in patients with COAD. Moreover, we found that ZEB2 has close relationship with ACTB, which was highly related to NETs. These findings suggest a dual role of ZEB2 in COAD growth, metastasis, and immunology.

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STATEMENT

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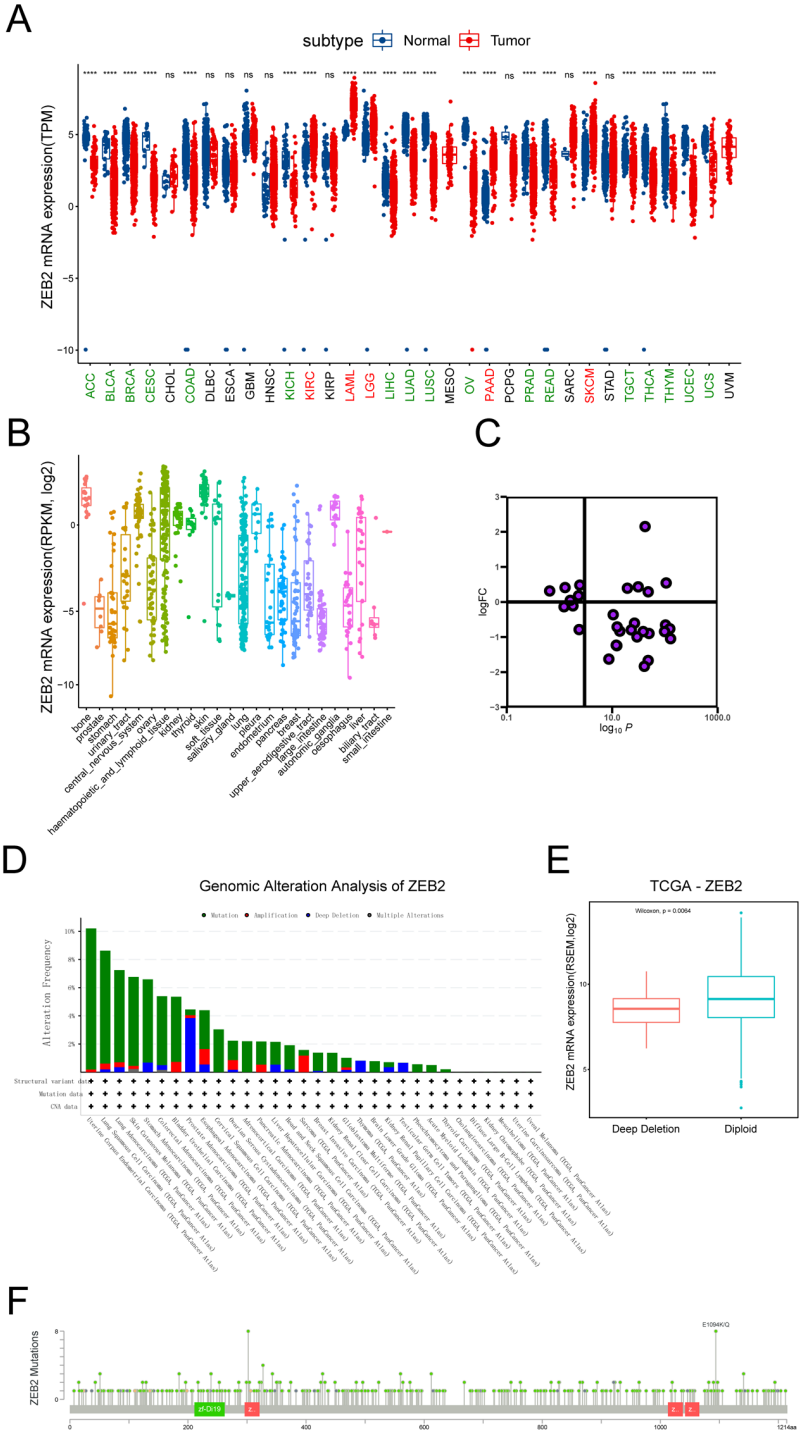
A
subtype
Normal
Tumor
ZEB2 mRNA expression(TPM)
ACC
BLCA
BRCA
CESC
CHOL
COAD
DLBC
ESCA
GBM
HNSC
KICH
KIRC
KIRP
LAML
LGG
LIHC
LUAD
LUSC
MESO
OV
PAAD
PCPG
PRAD
READ
SARC
SKCM
STAD
TGCT
THCA
THYM
UCEC
UCS
UVM
B
ZEB2 mRNA expression(RPKM, log2)
C
logFC
log10 P
D
Genomic Alteration Analysis of ZEB2
Mutation
Amplification
Deep Deletion
Multiple Alterations
Alteration Frequency
Structural variant data
Mutation data
CNA data
E
TCGA - ZEB2
Wilcoxon, p = 0.0064
ZEB2 mRNA expression(RSEM,log2)
Deep Deletion
Diploid
F
ZEB2 Mutations
E1094KQ

Figure 1

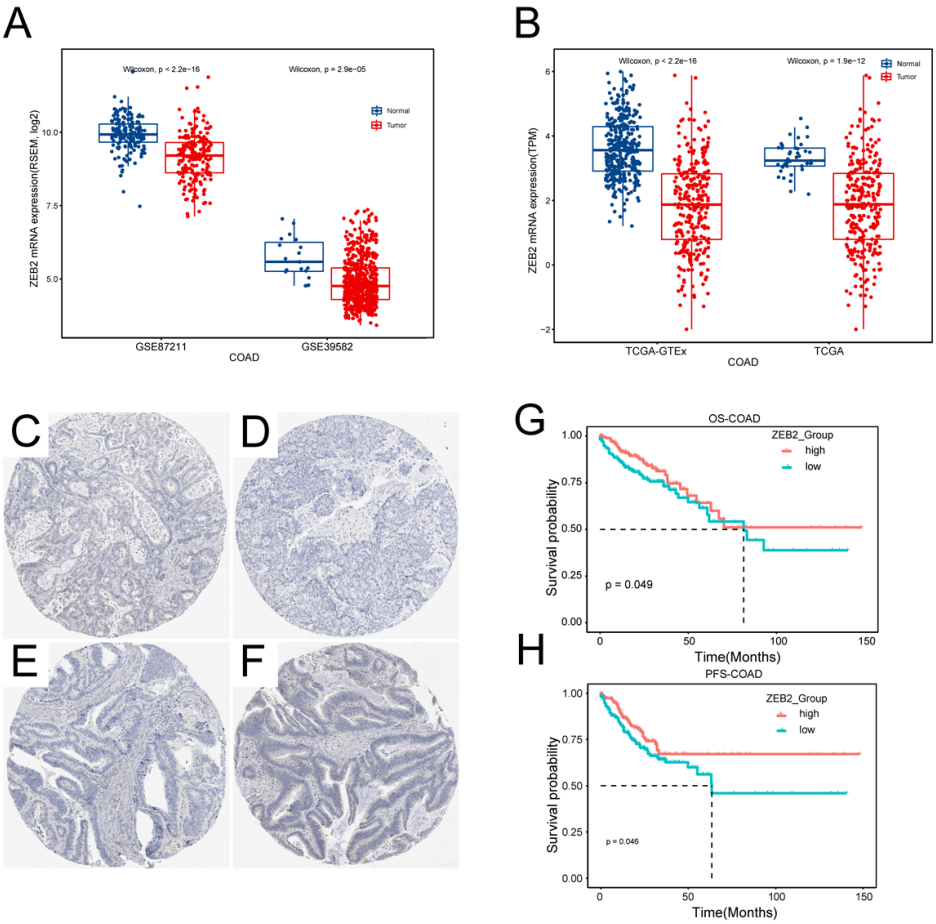
A
Wilcoxon, p < 2.2e−16
Wilcoxon, p = 2.9e−05
Normal
Tumor
ZEB2 mRNA expression(RSEM, log2)
10.0
7.5
5.0
GSE87211
GSE39582
COAD
B
Wilcoxon, p < 2.2e−16
Wilcoxon, p = 1.9e−12
Normal
Tumor
ZEB2 mRNA expression(TPM)
6
4
2
0
−2
TCGA-GTEx
TCGA
COAD
C
D
E
F
G
OS-COAD
ZEB2_Group
high
low
Survival probability
1.00
0.75
0.50
0.25
0.00
p = 0.049
0
50
100
150
Time(Months)
H
PFS-COAD
ZEB2_Group
high
low
Survival probability
1.00
0.75
0.50
0.25
0.00
p = 0.046
0
50
100
150
Time(Months)

Figure 2

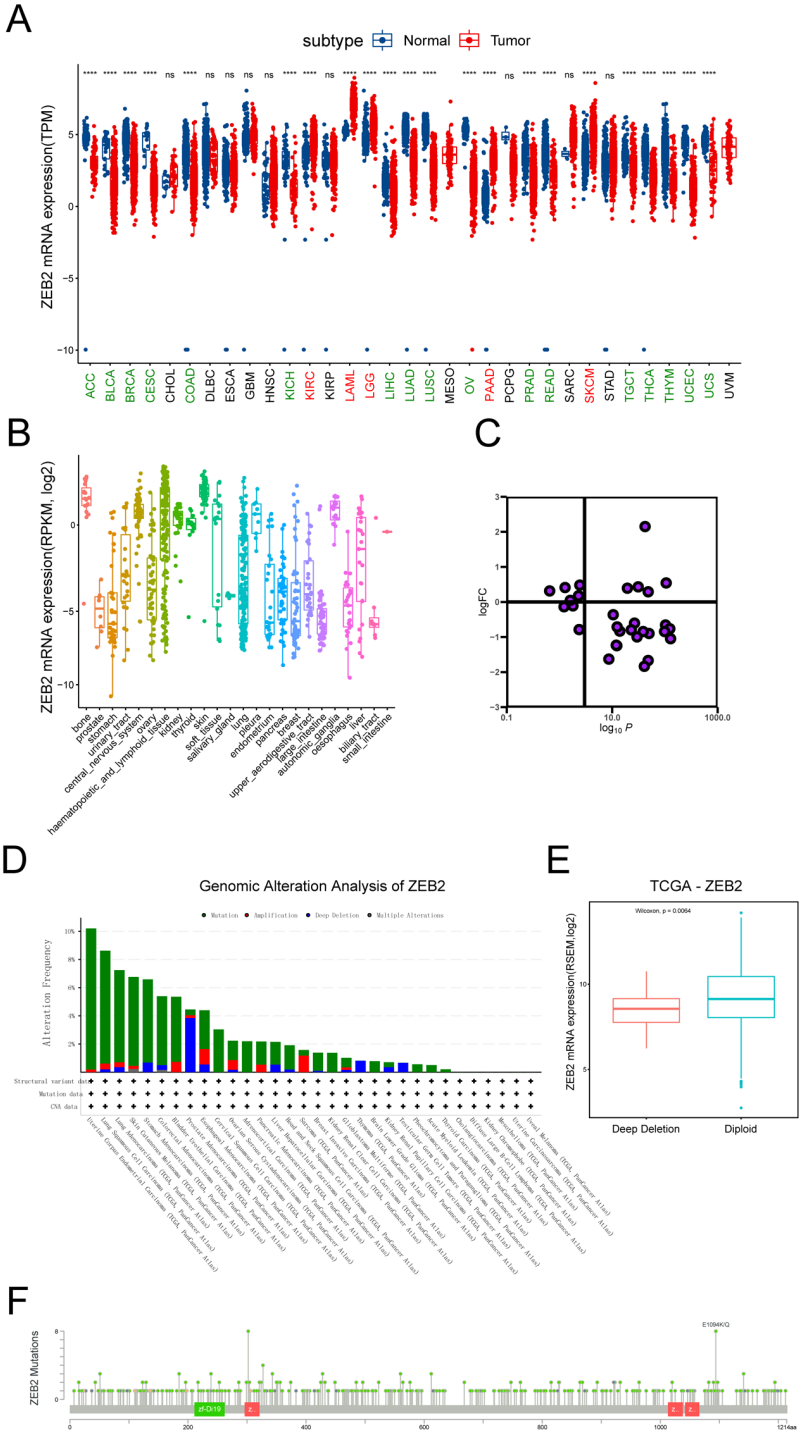
A
subtype
Normal
Tumor
ZEB2 mRNA expression(TPM)
ACC
BLCA
BRCA
CESC
CHOL
COAD
DLBC
ESCA
GBM
HNSC
KICH
KIRC
KIRP
LAML
LGG
LIHC
LUAD
LUSC
MESO
OV
PAAD
PCPG
PRAD
READ
SARC
SKCM
STAD
TGCT
THCA
THYM
UCEC
UCS
UVM
B
ZEB2 mRNA expression(RPKM, log2)
bone
prostate
stomach
urinary_tract
central_nervous_system
ovary
haematopoietic_and_lymphoid_tissue
kidney
thyroid
soft_tissue
skin
salivary_gland
lung
pleura
endometrium
pancreas
breast
upper_aerodigestive_tract
large_intestine
autonomic_ganglia
oesophagus
liver
biliary_tract
small_intestine
C
logFC
log10 P
D
Genomic Alteration Analysis of ZEB2
Mutation
Amplification
Deep Deletion
Multiple Alterations
Alteration Frequency
Structural variant data
Mutation data
CNA data
E
TCGA - ZEB2
Wilcoxon, p = 0.0064
ZEB2 mRNA expression(RSEM,log2)
Deep Deletion
Diploid
F
ZEB2 Mutations
E1094K/Q
pf-D/19
1214aa

Figure 3

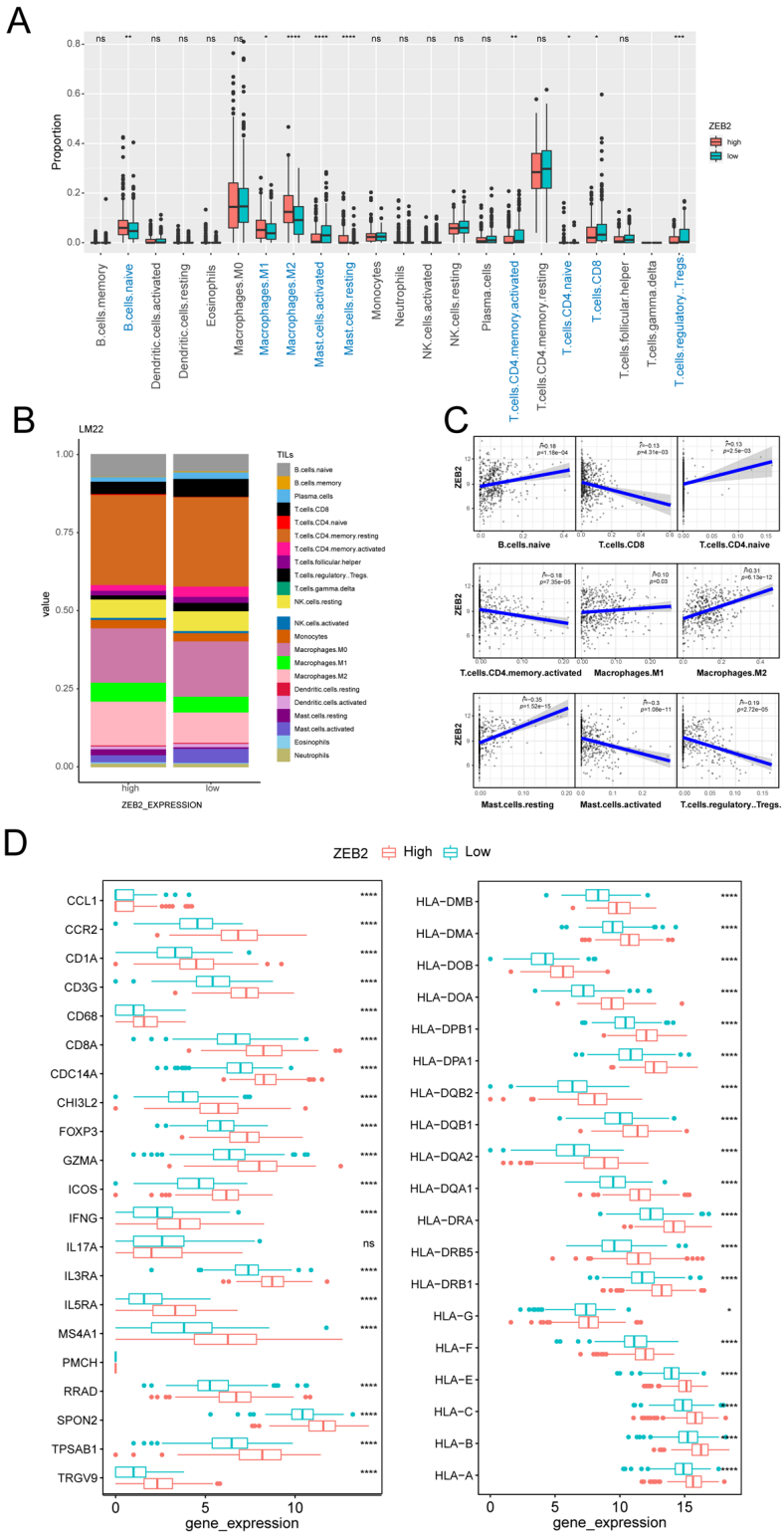
A
Proportion
ZEB2
high
low
B.cells.memory
B.cells.naive
Dendritic.cells.activated
Dendritic.cells.resting
Eosinophils
Macrophages.M0
Macrophages.M1
Macrophages.M2
Mast.cells.activated
Mast.cells.resting
Monocytes
Neutrophils
NK.cells.activated
NK.cells.resting
Plasma.cells
T.cells.CD4.memory.activated
T.cells.CD4.memory.resting
T.cells.CD4.naive
T.cells.CD8
T.cells.follicular.helper
T.cells.gamma.delta
T.cells.regulatory..Tregs.
B
LM22
value
TILs
high
low
ZEB2_EXPRESSION
C
ZEB2
B.cells.naive
T.cells.CD8
T.cells.CD4.naive
T.cells.CD4.memory.activated
Macrophages.M1
Macrophages.M2
Mast.cells.resting
Mast.cells.activated
T.cells.regulatory..Tregs.
D
ZEB2 High Low
CCL1
CCR2
CD1A
CD3G
CD68
CD8A
CDC14A
CHI3L2
FOXP3
GZMA
ICOS
IFNG
IL17A
IL3RA
IL5RA
MS4A1
PMCH
RRAD
SPON2
TPSAB1
TRGV9
HLA-DMB
HLA-DMA
HLA-DOB
HLA-DOA
HLA-DPB1
HLA-DPA1
HLA-DQB2
HLA-DQB1
HLA-DQA2
HLA-DQA1
HLA-DRA
HLA-DRB5
HLA-DRB1
HLA-G
HLA-F
HLA-E
HLA-C
HLA-B
HLA-A
gene_expression
gene_expression

Figure 4