Observational Study

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IKZF3 is a novel prognostic biomarker for head and neck squamous cell carcinoma

A study based on bioinformatics analysis

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Abstract

In the past few years, immunotherapy of tumors has become an extensive research hotspot, and the value of IKZF family genes in the tumor microenvironment has also been increasingly recognized. However, the expression of the IKAROS family zinc finger 3 (IKZF3) gene in human head and neck squamous cell carcinoma (HNSCC) and its prognostic value were not reported for the main subset until now. In the present study, we analyzed the relationship between IKZF3 gene expression and the survival of HNSCC patients. To evaluate the potential of IKZF3 as a prognostic biomarker for HNSCC comprehensively, multiple online analysis tools, including UALCAN, cBioPortal, GEPIA, WebGestalt, String, Genomic Data Commons, and TIMER databases were utilized in our study. We observed that the HNSCC patients with higher IKZF3 expression tended to exhibit longer overall survival. Univariate and multivariate Cox regression analyses indicated that age and grade were independent prognostic indicators in HNSCC. Moreover, Gene Ontology and KEGG function enrichment analyses showed that several pathways in HNSCC might be pivotal pathways regulated by IKZF3, which revealed that IKZF3 was probably participating in the occurrence and development of HNSCC. Furthermore, the hypomethylation of the IKZF3 gene was closely associated with genes that observed mutation in HNSCC. IKZF3 was significantly correlated with several immune cells in HNSCC (e.g., $CD8^+$ T cell, $CD4^+$ cell, and dendritic cell). We explored the potential prognostic values and roles of the IKZF3 in HNSCC, revealing that IKZF3 was probably a novel and reliable prognostic biomarker for patients with HNSCC.

Abbreviations: GO = Gene Ontology, HNSCC = head and neck squamous cell carcinoma, HPV = human papillomavirus, HR = hazard ratio, IKZF3 = IKAROS family zinc finger 3, MM = multiple myeloma, OS = overall survival, PPI = protein-protein interaction, TCGA = The Cancer Genome Atlas, TF = transcription factor, TIMER = Tumor Immune Estimation Resource.

Keywords: bioinformatics analysis, biomarker, IKZF3, head and neck squamous cell carcinoma, prognosis

1. Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy worldwide, with >550,000 new cases and over 380,000 deaths annually globally, with an incidence rate that has increased by 36.5% during the past decade. Approximately 95% of head and neck cancer cases are HNSCCs, which usually occur in the oral cavity, oropharynx, hypopharynx, and larynx mucosa.[1–3] The major risk factors for HNSCC are tobacco use, alcohol consumption, and infection with human papillomavirus (HPV).[4,5] Currently, conventional treatments for patients with HNSCC include surgery, radiotherapy, chemotherapy, and comprehensive treatment.[6] However, the majority of HNSCC cases are initially diagnosed at an advanced stage and respond poorly to conventional treatment. Although significant progress has been achieved in the treatment of HNSCC, the 5-year survival rate of patients with advanced stages (stages III and IV) is still below 50%.[7] Survival rates for various types of HNSCC have barely improved in the past 40 years.[8] Consequently, it is highly significant to screen a novel and reliable target to

HL, MY, ZH, and HL contributed equally to this work.

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The datasets generated during and/or analyzed during the current study are publicly available.

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prolong the overall survival (OS) and improve the quality of life of HNSCC patients.

IKAROS family zinc finger 3 (IKZF3), a member of the IKAROS family, has been revealed to play an essential role in the maturation of B and T cells. Aiolos is a zinc finger transcription factor (TF), encoded by IKZF3, that has a broad impact on gene expression patterns by activating chromatin modifiers like nucleosome remodeling and deacetylase complexes.[9,10] In more recent years, the crucial value of IKAROS family zinc finger members (IKZF) has been increasingly recognized in immune diseases and solid tumors,[11,12] and research in hematopoietic malignancies has been initially identified as evidence for the participation of the IKAROS family in carcinoma progression.[13,14] Mounting evidence suggests that the immune system plays a crucial role in tumorigenesis, growth, and metastasis, and immune-related genes are believed to be associated with tumor prognosis. we gather the following 3 aspects of evidence to explain the possibility of IKZF3 participating in the occurrence and development of HNSCC by regulating immune-related cells. Firstly, the 3 main and classical risk factors for HNSCC (e.g., tobacco, alcohol, and HPV) are associated with the tumor immune infiltration cells. Secondly, the head and neck region is enriched with lymph nodes and blood vessels, where activation of local immunity might act in restricting the spread of HNSCC and/or enhancing the response of HNSCC patients to treatment.[15] Finally, a targeted immune checkpoint pathway has been documented to have antitumor cytotoxic properties in refractory HNSCC. Given that regulatory abnormalities and mutations in IKZF family members have been identified in a range of cancers, including breast, lung, and colorectal cancers, we suggest that 3 major risk factors in HNSCC are associated with mutations in IKZF family members. It implies a link between immune response status and inflammatory processes affecting smokers, alcohol drinkers, or HPV-infected individuals. Therefore, new prognostic markers and therapeutic targets are urgently needed.

The advancement of HNSCC is typified by the accumulation of mutations in several oncogenes and oncogenes. There are 2 studies that confirm the previously reported mutation rates in HNSCC genes, including TP53, CDKN2A, PIK3CA, PTEN, HRAS, and NOTCH1.[16,17] Aiolos/IKZF3 is a member of the IKAROS family and is relevant to lung cancer.[18,19] Tobacco use is known as a major risk factor for nasopharyngeal carcinoma, and smoking status is strongly associated with treatment outcome, risk of recurrence, and survival. By analyzing epigenetic regulatory gene mutations in IKZF3 and HNSCC-related genes, IKZF3 smoking-related genes may become new molecular markers.

The possible role of IKZF3 in HNSCC has not been reported in the literature. The purpose of this research was to demonstrate the prognostic and predictive biomarker role of IKZF3 in HNSCC by bioinformatic analysis of clinical features and survival information from The Cancer Genome Atlas (TCGA). We also investigated the associated key signaling pathways. By analyzing a large amount of laboratory data published online, we focused on the IKZF3 gene expression, its altered features, and its potential value in prognosis.

2. Materials and methods

2.1. TCGA mRNA expression data

The TCGA analysis was performed by the National Cancer Institute and National Human Genome Research Institute to characterize the gene expression profile of >20,000 primary cancer samples and matched normal samples across 33 different cancer types.[19] There were 502 cases of primary head and neck squamous carcinoma (HNSCC), and 44 adjacent normal control samples. The clinical features, including gender, age, stage, and tumor grade, were included in the current study. Subsequently, the Genomic Data Commons Data Transfer Tool on the TCGA database was used to download the high-throughput sequencing fragments per kilobase of transcript per million mapped reads data of IKZF3.

2.2. Comparison of the mRNA expression of the IKZF3 in HNSCC and normal tissues

The differential expression of the IKZF3 in HNSCC in comparison to healthy tissues was analyzed using limma package in R 3.6.0 software, and Perl 5.26 software was used to obtain the mRNA expression levels of the IKZF family from the high-throughput sequencing level 3 data on genome mRNA expression.

2.3. Analysis of TCGA dataset using UALCAN

The HNSCC data in the TCGA database were analyzed using the UALCAN platform (http://ualcan.path.uab.edu). UALCAN is an online open-access platform that contains TCGA raw data, including gene expression and clinicopathological data.[20] In the present study, the UALCAN database was employed to analyze the relationship with IKZF3 in clinicopathological parameters (age, stage, and grade) and expression in HNSCC tissues. Afterward, we utilized the platform to further understand the correlation between methylation level and IKZF3 expression in HNSCC. Besides, samples lacking cancer stage or grade information were excluded from the corresponding analyses.

2.4. Analysis of genetic mutations in the IKZF family using cBioPortal

In the present study, genetic mutations in the IKZF family and their correlation with OS and progression-free survival of HNSCC patients were explored. The cBioPortal database (http://www.cbioportal.org) was utilized to analyze the mutation rate of IKZF family members.[21]

2.5. Correlation among HNSCC-related genes and immune infiltrates analysis with IKZF3 using the TIMER

We performed Pearson correlation analysis to determine the gene correlation between IKZF3 and HNSCC-related genes by using the Tumor Immune Estimation Resource tool (TIMER, https://cistrome.shinyapps.io/timer/). And TIMER is an online tool for systematical analyses of immune infiltration of various cancers. In this study, the purity-corrected partial Spearman correlation (partial-cor) and *P* value provided by TIMER were shown in scatterplots.[22]

2.6. Functional enrichment analyses of IKZF3 using WebGestalt

The web version WebGestalt (http://www.webgestalt.org) supported 12 organisms, 354 gene identifiers, and 321,251 function categories.[23] Three well-established and complementary methods for enrichment analysis were available, including the Over-Representation Analysis, Gene Set Enrichment Analysis, and Network Topology-based Analysis. In addition to the Over-Representation Analysis, WebGestalt also supported Gene Set Enrichment Analysis and Network Topology-based Analysis. An interactive and efficient exploration of enrichment analysis could be performed using WebGestalt with similar gene identifiers.

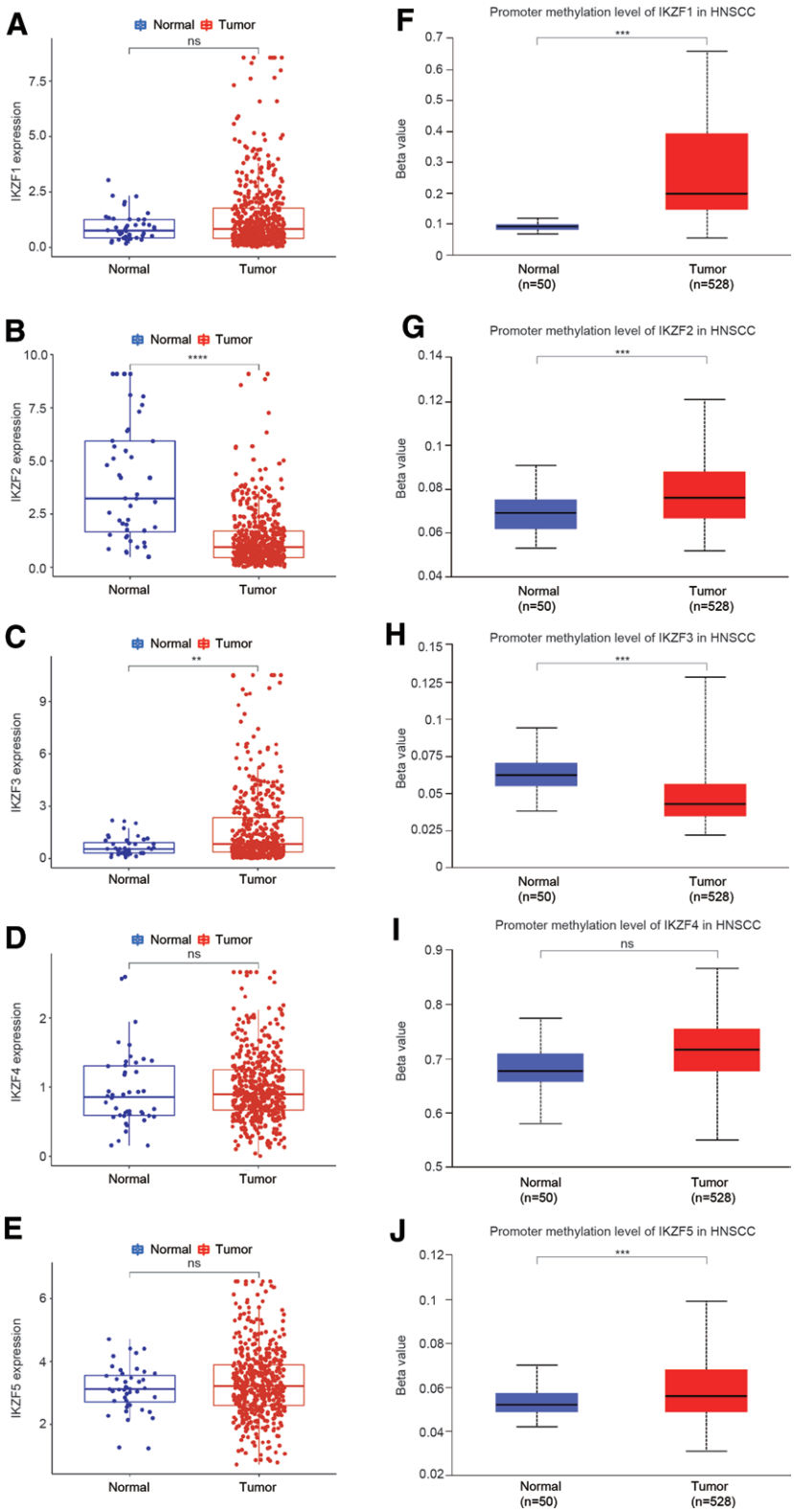

Figure 1. (A–E) Expression and methylation level of IKZF family. Compared with correspondingly normal tissues, (B) IKZF2 is down-expressed and (C) IKZF3 is up-expressed in tumor tissues, while there is no statistical difference in expression of (A, D, E) others in the IKZF family between tumor and normal tissue. (F–J) The hypermethylation of (F, G, J) IKZF1/2/5 was observed, and the hypomethylation was seen in (H) IKZF3. IKZF3 = IKAROS family zinc finger 3.

2.7. Protein-protein interaction (PPI) network construction

Similar genes that have a similar expression pattern with IKZF3 in HNSCC were obtained from GEPIA (http://gepia.cancer-pku.cn). Besides, the relationship between IKZF3 and 6 tumor-related genes is analyzed with GEPIA. The top 100 similar genes were subjected to PPI analysis in STRING (WebGestalt).[24]

2.8. Pan-caner analysis based on TCGA

The 33 tumor patients in the TCGA database, tumor RNA-seq data (TCGA), and IKZF3 can be downloaded from the Genomic Data Commons (https://portal.gdc.cancer.gov/). Each tumor has mRNA expression data and miRNA expression data of a paired normal tissue sample. Use R software v4.0.3 for statistical analysis.

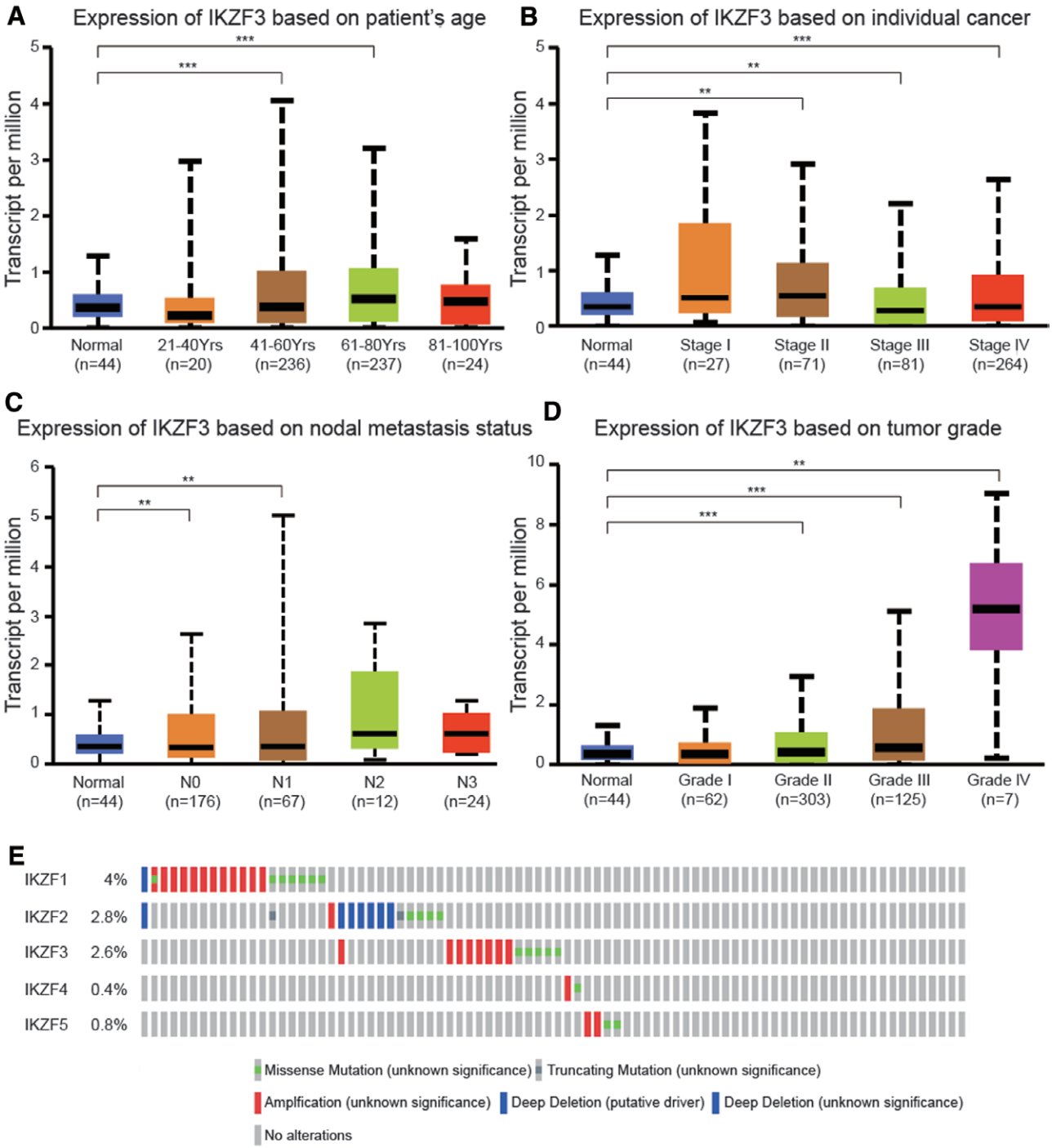

Figure 2. (A–D) IKZF3 is a robust biomarker for patients with HNSCC. (E) Statistically significant differences were observed between tumor and normal samples grouped based on clinical data such as age, tumor stage, lymph node metastasis, and tumor grade. IKZF1/2/3 is high-frequency mutated in HNSCC patients. HNSCC = head and neck squamous cell carcinoma.

2.9. The survival analysis of the IKZF3 in HNSCC

The correlation between the mRNA expression of IKZF3 with the prognosis of HNSCC patients was evaluated using R package survival analysis, the OS rate, and the best optimal cutoff value was identified. Moreover, a nomogram is produced based on multivariate Cox analyses to assess the influence on patient survival.

2.10. Statistical methods

Differences between the 2 groups were compared by using the Student *t* test. Correlations were determined using Pearson or Spearman correlation tests, as appropriate. The survival curve was plotted by the KM method, with a hazard ratio (HR) with 95% confidence intervals and log-rank *P* value. **P* < .05, ***P* < .01, ****P* < .001 were considered statistically significant difference in all circumstances. If not otherwise stated, the rank sum test detects 2 sets of data, and a *P* value of <.05 is considered statistically significant.

3. Results

3.1. IKZF3 expression in HNSCC patients

In the present study, TCGA data of HNSCC were utilized to validate the expression patterns of the IKZF members. The results of

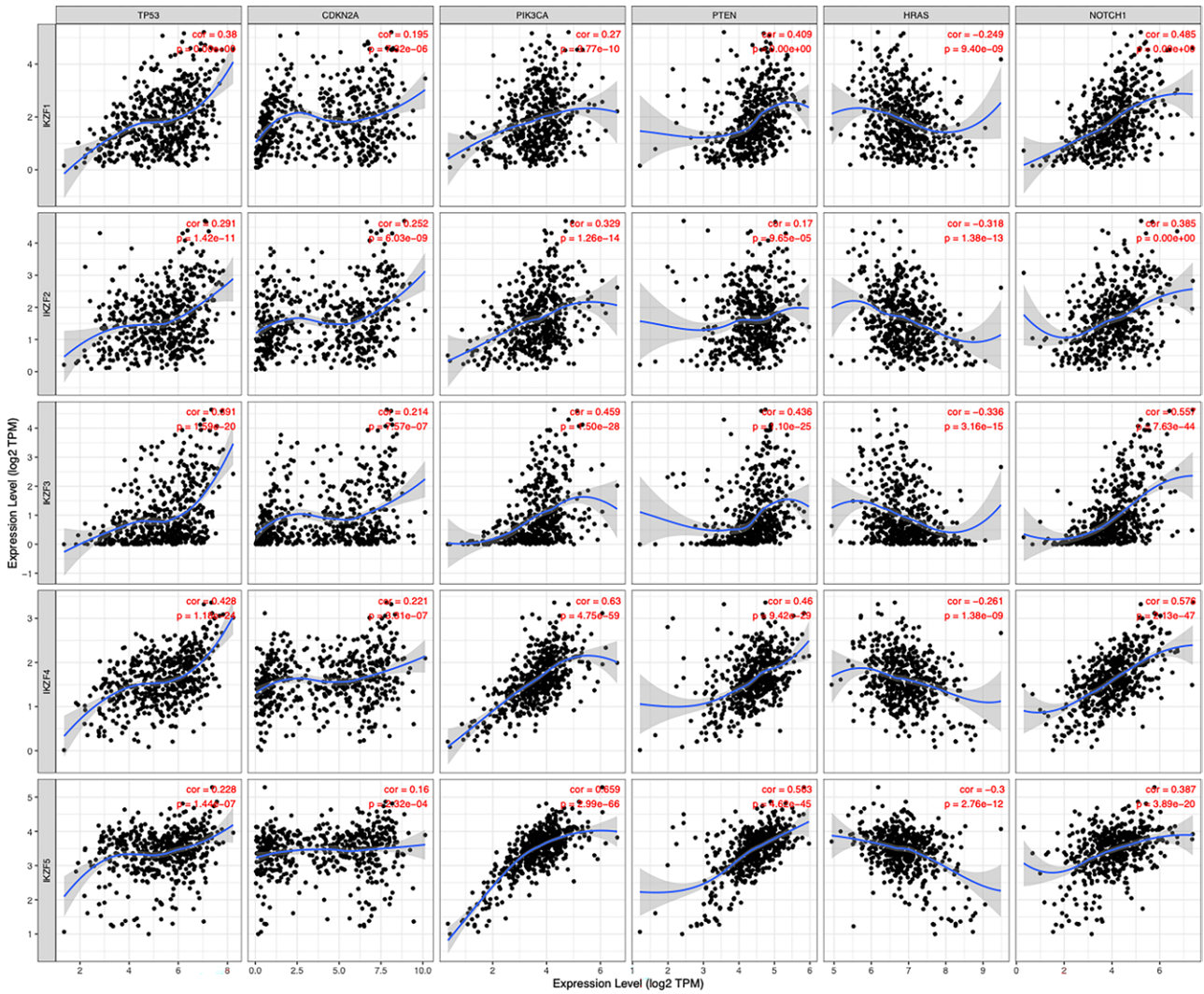

Figure 3. IKZF family is closely related to 6 genes related to HNSCC. IKZF family is positively correlated with TP53, CDKN2A, PIK3CA, PTEN, HRAS, and NOTCH1. HNSCC = head and neck squamous cell carcinoma.

UALCAN revealed that the expression levels of IKZF2/3 were significantly different among IKZF members between tumor and normal tissues in HNSCC. IKZF3 was remarkably higher as compared with normal samples, while IKZF2 exhibited a relatively lower expression level (Fig. 1A–J). By UALCAN analysis of samples, statistically significant differences were observed between tumor and normal samples grouped based on clinical data such as age, tumor stage, lymph node metastasis, and tumor grade (Fig. 2A–D). These results suggest that the upregulation of IKZF3 may be closely associated with the biological characteristics of HNSCC.

3.2. DNA methylation of IKZF members in HNSCC

DNA methylation levels of IKZF family members with the prognostic value of each single CpG were investigated by the UALCAN tool. As shown in Figure 1B, box plots showed that the hypermethylation of IKZF1/2/5 was observed, and the hypomethylation was seen in the IKZF3.

3.3. Genetic alteration and neighbor gene network of IKZF3 in HNSCC

Genetic alterations in IKZF members were explored by the cBioPortal database. In our research, 530 HNSCC patients with IKZF gene mutation information from the TCGA dataset were analyzed. The differential degrees of genetic variation among IKZF family members are shown in Figure 2E. Pan-Cancer Atlas Studies defined mutation types into 4 components missense mutations, truncating mutations, inframe mutations, and other mutations, which were colored with respect to the corresponding mutation types. The percentages of genetic alterations in HNSCC of IKZF members ranged from 0.8 to 4% for single genes (IKZF1, 4%; IKZF2, 2.8%; IKZF3, 2.6%; IKZF4, 0.4%; IKZF5, 0.8%). By using the TIMER database, we compared the association between IKZF family members and 6 HNSCC-related genes including TP53, CDKN2A, PIK3CA, PTEN, HRAS, and NOTCH1 (Fig. 3). It showed that IKZF family members were associated with the 6 genes related in HNSCC. Presented positive and significant correlations were found among IKZF3/4/5 and TP53, CDKN2A, PIK3CA, PTEN, and NOTCH1. Negative correlations were found in IKZF1-5 and HRAS.

Based on the results of the expression and survival analysis described above, we selected IKZF3 for further bioinformatics analysis. The top 100 genes similar to the IKZF3 gene were detected in HNSCC with GEPIA. A PPI (Fig. 4) network was generated in the STRING protein interaction database.

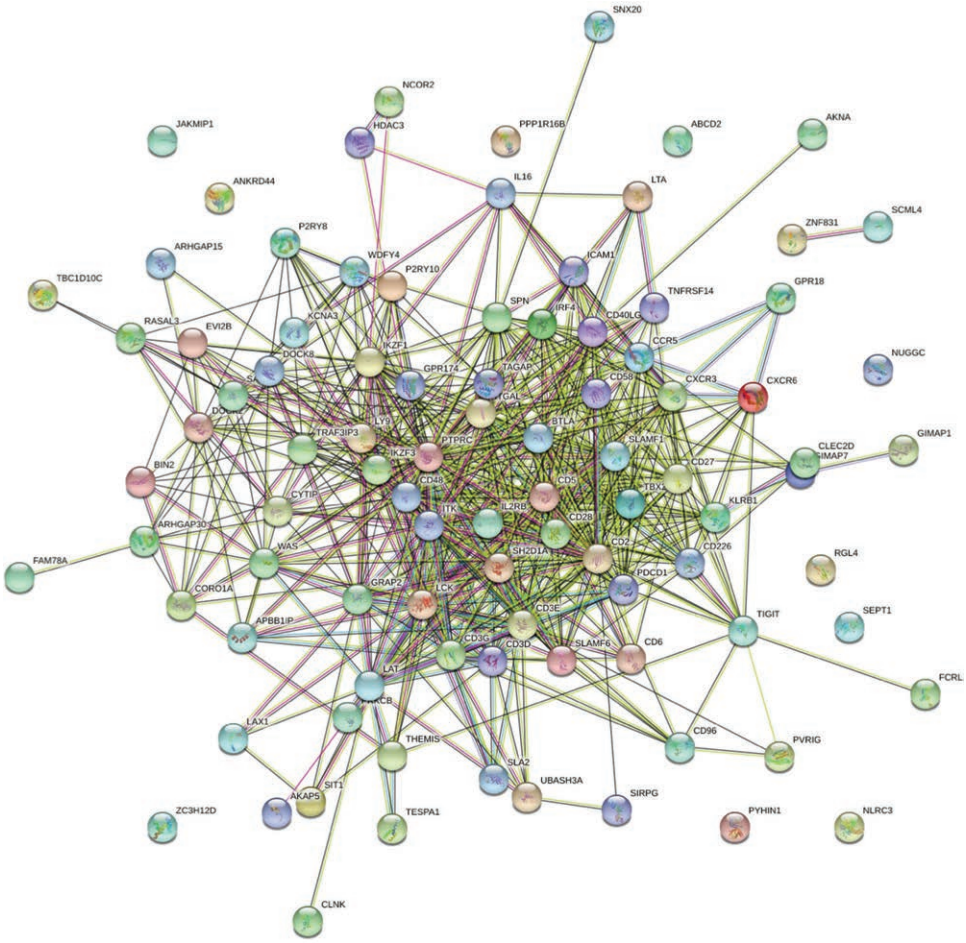

Figure 4. The protein-protein interaction network in the STRING protein interaction database. The PPI interaction network visualized the relationship between IKZF3 and genes related to IKZF3. IKZF3 = IKAROS family zinc finger 3, PPI = protein-protein interaction.

3.4. Functional and pathway enrichment analyses of IKZF3 in HNSCC

Additionally, the top 100 similar genes of IKZF3 were obtained (Table 1) by GEPIA. The biological functions of IKZF3 and similar genes were explored by Gene Ontology (GO) annotation and KEGG pathway analyses in the WEB-based GEne SeT AnaLysis Toolkit (http://www.webgestalt.org/option.php). GO described our knowledge of the biological domain in 3 aspects: biological process, cellular component, and molecular function. Functional annotation enrichment analysis using GO and KEGG pathway. GO analysis showed that similar genes were significantly enriched in biological processes such as biological regulation, response to stimulus and cell communication, molecular functions such as protein binding and ion binding, and cellular components such as membrane, cytosol, and vesicle (Fig. 5A–C). In functional database, we also performed KEGG analysis to define the pathways showing positive correlations between IKZF3 and the most frequently altered neighbor genes (Fig. 5E). Enrichment results revealed that hsa04660 (T cell receptor signaling pathway), hsa04514 (Cell adhesion molecules), hsa04659 (Th17 cell differentiation), hsa04658 (Th1 and Th2 cell differentiation) were primarily involved (Fig. 5D).

3.5. Tumor-infiltrating immune cells associated with IKZF3

Last but not least, we also studied the potential immunological correlation between IKZF3 and tumor-infiltrating immune cells (Fig. 5F). The expression level of IKZF3 was negatively correlated with tumor purity ($P < .05$), suggesting that IKZF3 was highly expressed in the HNSCC microenvironment. The association between IKZF3 and immune cells in HNSCC was comparably high, B cell (partial-cor = 0.411, $P = 7.86E-21$), $CD8^+$ T cell (partial-cor = 0.57, $P = 3.35E-42$), $CD4^+$ T cell (partial-cor = 0.631, $P = 8.88E-55$), macrophage (partial-cor = 0.475, $P = 1.31E-28$), neutrophil (partial-cor = 0.537, $P = 3.76E-37$) and dendritic cell (partial-cor = 0.649, $P = 5.15E-59$).

3.6. Prognostic values of IKZF3 in HNSCC

We assessed the prognostic values of IKZF3 expression in patients with HNSCC with the Prognosis. Combined with significantly higher expression levels of IKZF3 shown above, we analyzed the association between IKZF3 and OS. As shown in Figure 6A, IKZF3 was associated with better higher among tumors than normal tissues. It suggested that IKZF3 showed significant prognostic values.

So far, the IKZF3 has been identified in various cancers, apart from HNSCC, breast cancer, colon cancer, kidney cancer, lung cancer, etc were included. To develop a clinically applicable method that could predict the survival probability of a patient, we analyzed the univariate and multivariate Cox proportional hazards and found that IKZF3 ($P = .00847$, HR = 0.85) and age ($P = .00097$, HR = 1.02), and stage ($P = .00009$, HR = 1.4) were significantly different between the non-recurrence and recurrence groups (Fig. 6B and C). It showed that age, stage, and IKZF3 expression were found to be the independent factors affecting patient survival, which corresponded with the analysis shown above. A nomogram was developed based on the results of the multivariate Cox

Table 1

The list of the top 100 similar genes of IKZF3 obtained by GEPIA.

Gene symbol	Gene ID	PCC
NLRC3	ENSG00000167984.16	0.90
ZNF831	ENSG00000124203.5	0.90
UBASH3A	ENSG00000160185.13	0.89
CD6	ENSG00000013725.14	0.88
SLAMF6	ENSG00000162739.13	0.88
ITGAL	ENSG00000005844.17	0.88
IKZF1	ENSG00000185811.16	0.87
AKNA	ENSG00000106948.16	0.87
PPP1R16B	ENSG00000101445.9	0.87
SH2D1A	ENSG00000183918.14	0.86
SASH3	ENSG00000122122.9	0.86
GPR174	ENSG00000147138.1	0.86
CD3E	ENSG00000198851.9	0.86
TRAC	ENSG00000277734.4	0.85
PVRIG	ENSG00000213413.2	0.85
P2RY8	ENSG00000182162.9	0.85
P2RY10	ENSG00000078589.12	0.84
LINC00426	ENSG00000238121.5	0.84
GVINP1	ENSG00000254838.5	0.84
IL16	ENSG00000172349.16	0.84
CD2	ENSG00000116824.4	0.83
ITK	ENSG00000113263.12	0.83
IL2RB	ENSG00000100385.13	0.83
ARHGAP30	ENSG00000186517.13	0.83
TIGIT	ENSG00000181847.11	0.83
SIRPG	ENSG00000089012.14	0.83
TESPA1	ENSG00000135426.14	0.83
SLAMF1	ENSG00000117090.14	0.83
RP1-47M23.3	ENSG00000280135.1	0.83
GRAP2	ENSG00000100351.16	0.82
LY9	ENSG00000122224.17	0.82
TRBV25-1	ENSG00000211751.7	0.82
TRBC2	ENSG00000211772.8	0.82
CXCR3	ENSG00000186810.7	0.82
CD3G	ENSG00000160654.9	0.82
RP11-284N8.3	ENSG00000259834.1	0.82
CLEC2D	ENSG00000069493.14	0.82
TRBV5-1	ENSG00000211734.3	0.81
SEPT1	ENSG00000180096.11	0.81
CD27	ENSG00000139193.3	0.81
PYHIN1	ENSG00000163564.14	0.81
GPR18	ENSG00000125245.12	0.80
RASAL3	ENSG00000105122.12	0.80
SCML4	ENSG00000146285.13	0.80
PTPRC	ENSG00000081237.18	0.80
SIT1	ENSG00000137078.8	0.80
KLRB1	ENSG00000111796.3	0.80
BTLA	ENSG00000186265.9	0.80
RGL4	ENSG00000159496.14	0.80
NUGGC	ENSG00000189233.11	0.80
WAS	ENSG00000015285.10	0.80
LTA	ENSG00000226979.8	0.79
CXCR6	ENSG00000172215.5	0.79
CD96	ENSG00000153283.12	0.79
ARHGAP15	ENSG00000075884.12	0.79
EVI2B	ENSG00000185862.6	0.79
CORO1A	ENSG00000102879.15	0.79
KCNA3	ENSG00000177272.8	0.79
CD48	ENSG00000117091.9	0.79
PDCD1	ENSG00000188389.10	0.79
FTH1P22	ENSG00000225079.2	0.79
SPN	ENSG00000197471.11	0.79
LCK	ENSG00000182866.16	0.79
LAT	ENSG00000213658.10	0.78
PRKCB	ENSG00000166501.12	0.78
APBB1IP	ENSG00000077420.15	0.78
SNX20	ENSG00000167208.14	0.78
TRAF3IP3	ENSG00000009790.14	0.78
ANKRD44	ENSG00000065413.16	0.78

Table 1

(*Continued*)

Gene symbol	Gene ID	PCC
SLA2	ENSG00000101082.13	0.78
BIN2	ENSG00000110934.10	0.78
JAKMIP1	ENSG00000152969.16	0.78
TBC1D10C	ENSG00000175463.11	0.78
CLNK	ENSG00000109684.14	0.77
AKAP5	ENSG00000179841.8	0.77
TRBV20-1	ENSG00000211747.3	0.77
LAX1	ENSG00000122188.12	0.77
IRF4	ENSG00000137265.14	0.77
TRBV2	ENSG00000226660.2	0.77
CYTIP	ENSG00000115165.9	0.77
BZRAP1-AS1	ENSG00000265148.5	0.77
DOCK2	ENSG00000134516.15	0.77
CD28	ENSG00000178562.17	0.77
GIMAP7	ENSG00000179144.4	0.76
TBX21	ENSG00000073861.2	0.76
WDFY4	ENSG00000128815.17	0.76
FCRL3	ENSG00000160856.20	0.76
CCR5	ENSG00000160791.13	0.76
THEMIS	ENSG00000172673.10	0.76
ZC3H12D	ENSG00000178199.13	0.76
TRBV29-1	ENSG00000232869.2	0.76
AC006129.2	ENSG00000268027.5	0.76
DOCK8	ENSG00000107099.15	0.76
RP11-75L1.2	ENSG00000213443.2	0.76
TAGAP	ENSG00000164691.16	0.76
TRAV8-6	ENSG00000211795.3	0.76
TRBV10-3	ENSG00000275791.1	0.76
RP11-1094M14.5	ENSG00000267074.1	0.75
CD226	ENSG00000150637.8	0.75
PCED1B-AS1	ENSG00000247774.6	0.75

IKZF3 = IKAROS family zinc finger 3.

proportional hazards analysis, it predicts 1-, 2-, 3-, and 5-year OS of HNSCC patients (Fig. 6D). The total score was 0 to 220, and each variable was calculated and merged. A high score indicates a high risk of recurrence and a low risk of recurrence-free probability. According to the correction curve, we discovered that the nomogram in 3 years exhibited the best predictive ability (Fig. 6E).

4. Discussion

IKZF3 is a member of the IKAROS family and plays an important role in the maturation of B and T cells,[9,10] it has been reported in hematopoietic malignancies, immunological diseases, and solid tumors.[11–14] However, the association of IKZF3 expression with HNSCC has not been reported. This is the first study to explore the prognostic value of IKZF3 expression in HNSCC. Our findings add to the current knowledge and may contribute towards improving treatment options and increasing the accuracy of prognosis for patients with HNSCC.

The IKAROS zinc finger (IKZF) protein family consists of 5 TFs (IKAROS, Helios, Aiolos, Eos, and Pegasus or IKZF1-IKZF5) with a well-documented role in lymphocyte development and differentiation. IKZF3 is one of the members of the IKAROS family, which plays a critical role in the development of B and T cells. It has been demonstrated that the IKZF3 gene is dramatically correlated with hematopoietic malignancies such as multiple myeloma (MM) and chronic lymphocytic leukemia. IKZF3 protein expression levels were remarkably elevated in patients with MM stage III and correlated with higher OS.[25] Hung et al reported that IKZF3 collaborates with Blimp-1 to regulate the survival of MM cells.[26] Elevated IKZF3 expression has been reported to promotes cell survival by regulation of Bcl2 family proteins in chronic lymphocytic leukemia.[27] Furthermore, several studies have demonstrated that multiple IKZF3 genes are of significant relevance to Grave disease,[28] systemic lupus erythematosus,[29,30] rheumatoid arthritis,[31] asthma,[32] primary biliary cirrhosis,[33] etc indicating its comprehensive involvement with autoimmune or immunological diseases. Over the past decade, the tumor microenvironment, a complex ecosystem actively involved in all stages of cancer initiation and progression, has been extensively studied and consists of a complex immune cell system including T cells, B cells, dendritic cells, macrophages, and NK cells.[34] IKAROS family members are gaining involvement in solid tumors, and IKZF3 is compositionally upregulated in certain cancers and is strongly associated with poor prognosis. Sharma et al[35] reported that IKZF1 promotes metastatic ability through the upregulation of Slug and matrix metalloproteinase 2 in ovarian cancer. The IKZF1 gene is associated with a higher risk of (colorectal cancer) CRC within the population of Jammu and Kashmir.[36] Overexpression of IKZF3 promotes epithelial-mesenchymal transition and cancer stem cell-like properties in lung cancer cells.[37] Ectopic expression of Aiolos in the breast cancer cell line BT474 results from a fusion event of its transcriptional control by the VABP promoter.[38] IKZF3 overexpression was a prognostic factor of worse survival in patients with NSCLC.[39] IKZF2 was found in laryngeal squamous cell carcinoma, as the hub transcriptional regulator of immune-related differentially expressed genes.[40] However, the significance of IKZF3 expression in prognosis in patients with HNSCC is largely unclear.

In our study, we analyzed the IKZF3 expression profile in numerous human solid tumors in the TCGA database. The results demonstrated that IKZF3 gene expression was comparably higher in HNSCC, breast cancer, colon cancer, kidney cancer, lung cancer, and others in their matched adjacent normal

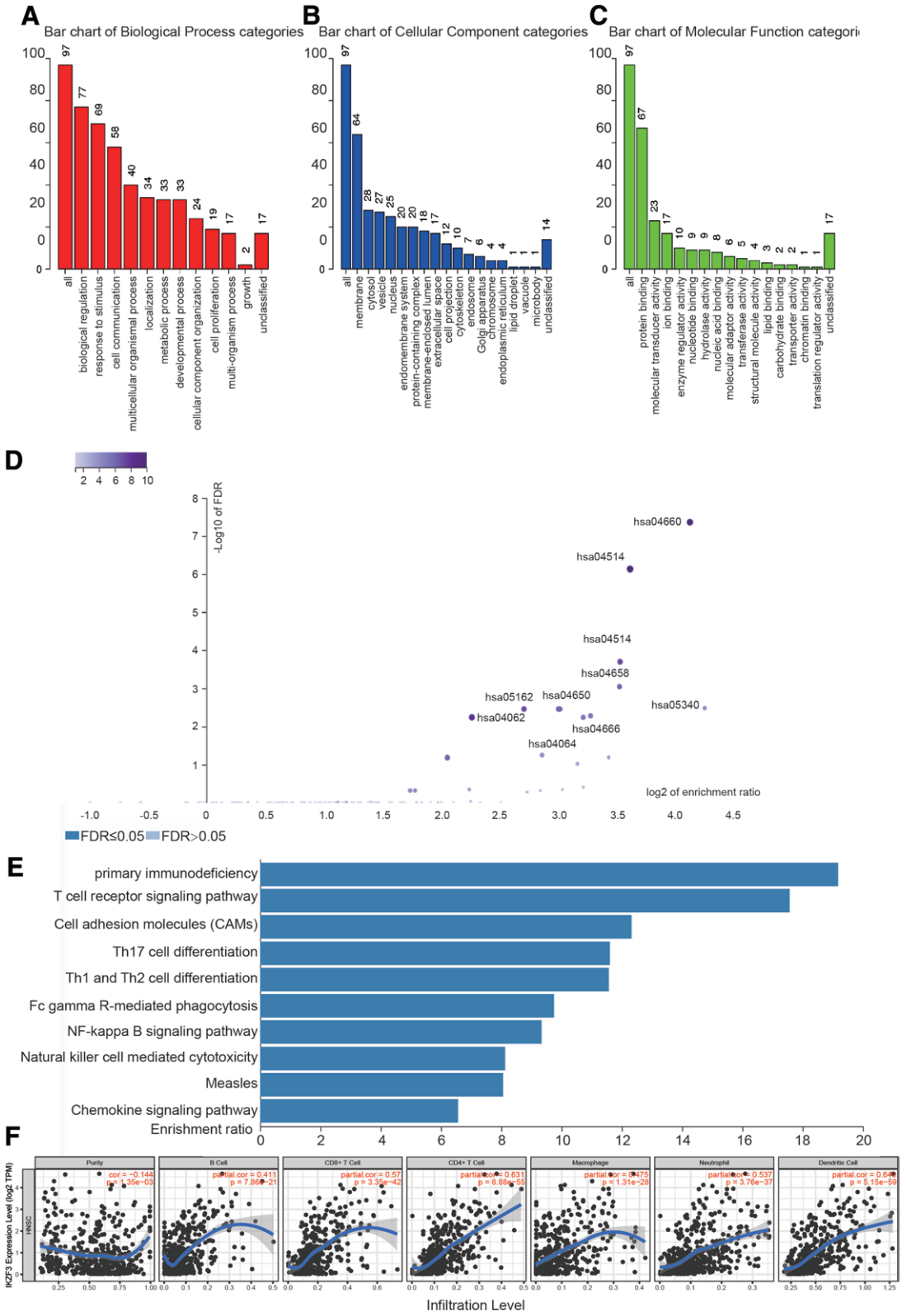

Figure 5. The function enrichment of genes related to IKZF3. (A–C) Similar genes were significantly enriched in biological processes such as biological regulation, response to stimulus and cell communication, molecular functions such as protein binding and ion binding, and cellular components such as membrane, cytosol, and vesicle. (D) The hsa04660 (T cell receptor signaling pathway), hsa04514 (Cell adhesion molecules), hsa04659 (Th17 cell differentiation), and hsa04658 (Th1 and Th2 cell differentiation) were primarily involved. (E) Several pathways were identified that positively correlated between IKZF3 and the most frequently altered neighbor genes. (F) The potential immunological correlation between IKZF3 and tumor-infiltrating immune cells was explored. IKZF3 = IKAROS family zinc finger 3.

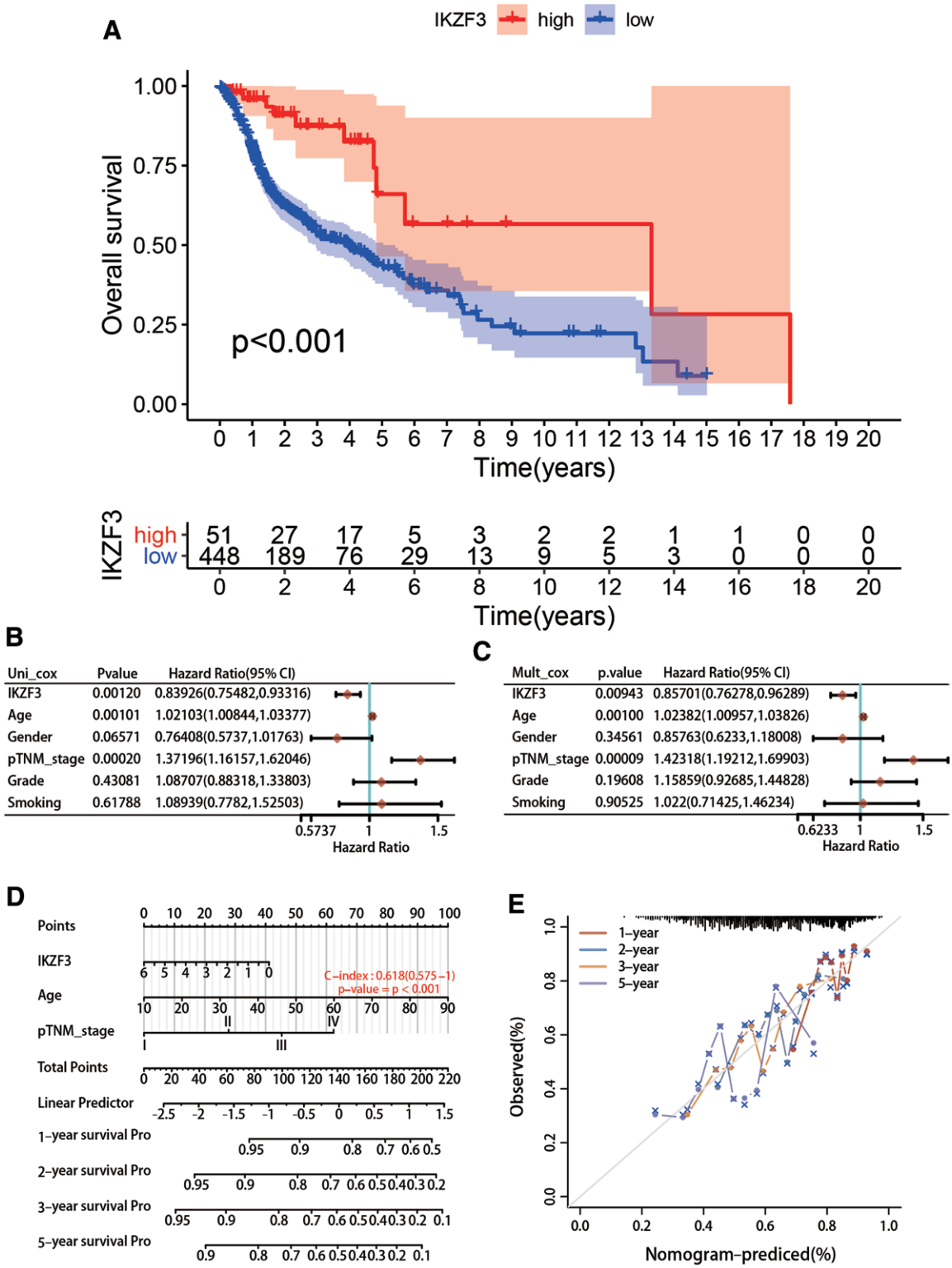

Figure 6. IKZF3 could act as a reliable prognostic indicator for patients with HNSCC. (A) Patients with IKZF low-expressed exhibited survival advantage in OS. (B–C) IKZF3, age, and stage were significantly different between the non-recurrence and recurrence groups. (D) The nomogram revealed the risk-scoring criteria of patients with HNSCC. (E) The nomogram in 3 years exhibited the best predictive ability. HNSCC = head and neck squamous cell carcinoma, IKZF3 = IKAROS family zinc finger 3, OS = overall survival.

tissues. IKZF3 gene expression and its potential prognostic impact on patients with HNSCC have not been evaluated. In 2015, Li et al explored the molecular and clinical consequences of Aiolos expression by lung cancers.[39] The results revealed that the hematopoietic lineage protein Aiolos is frequently expressed in lung cancers and its high expression level correlates with extremely poor survival rates in human lung cancer and did not correlate with age, TNM status, smoking history, or sex. In the present study, we evaluated the IKZF3 gene expression profile via bioinformatic analysis using the TCGA database. With

respect to matched normal tissues, IKZF3 gene expression levels were significantly higher in patients with HNSCC. However, the survival analysis revealed that patients with IKZF3 high expression had better OS ($P < .001$). Univariate and multivariate Cox analysis further confirmed that IKZF3 high expression was associated with better OS based on independent risk factors (age and tumor stage) in patients with HNSCC; other clinicopathologic features were also analyzed in HNSCC, there were significant differences between tumor and normal samples grouped in age, tumor stage, race, gender, grade, lymph node metastasis, and TP53-mutation status by using the UALCAN database. At present, a predictive nomogram for HNSCC by combining the expression value of IKZF3 with clinical variables has not been reported. Therefore, we constructed a prognostic nomogram by integrating clinical factors and gene expression via the TCGA dataset to enable clinicians to predict the risk of individual patient death and guide patient assessment and therapeutic decision-making. We found that the high IKZF3 expression phenotype was associated with age, pTMN stage, and OS, which further confirms our analysis shown above. These results suggest that the overexpression of IKZF3 could be a novel potential prognostic marker in HNSCC.

DNA methylation, namely the covalent addition of a methyl group (CH3) to carbon in the 5 positions of cytosine in the sequence 5′-CG-3′, represents one of the most common epigenetic mechanisms.[41,42] In normal mammalian cells long interspersed nuclear element sequences have a high methylation status, while during cancer development they are hypomethylated, which contributes to activating transcription of sequences that influence genome instability and as a result may facilitate carcinogenesis.[31] Li et al concluded that the overall DNA hypomethylation level is associated with a poorer prognosis.[43] Hypomethylation in promoter regions of genes has been shown in several studies of HNSCC.[44–46] The impact of overexpression of IKZF3 induces hypomethylation is an important process to regulate gene expression in HNSCC, which could be connected with chromosomal instability and gene activation in some ways. As we can see, hypomethylation of IKZF3 is shown above in HNSCC (2.6%). It is known that DNA methylation changes can lead to the development of mutations and also mutations can cause altered DNA methylation.[47] We selected 6 genes previously reported associated with HNSCC, including TP53, CDKN2A, PIK3CA, PTEN, HRAS, and NOTCH1. Notably, the IKZF3 gene was related to those genes ($P < .001$) by TIMER. This result prompted us to draw a hypothesis that IKZF3 plays an important role in HNSCC. But it still needs some evidence to prove by experimental data.

We mapped the top 100 similar genes with IKZF3 into the STRING database and obtained the PPI network, to identify the interactions among them. Functional enrichment and analysis were carried out to further understand the role of similar genes with IKZF3 in HNSCC. The GO enrichment analysis results indicated that these genes are primarily involved in biological processes such as biological regulation, response to stimulus, and cell communication. Furthermore, KEGG pathway enrichment analysis revealed that similar genes were enriched in multiple pathways including, the T cell receptor signaling pathway, Cell adhesion molecules, Th17 cell differentiation, and Th1 and Th2 cell differentiation. It is well-documented that those pathways play a key role in cancer invasion and metastasis.[48,49] Thus, our findings show that IKZF3 may be involved in the invasion and metastasis of HNSCC.

The immune system has been considered to affect the development, growth, and metastasis of tumors. Recent studies reported that the IKZF3 gene has a significant association with immune-related diseases including Grave disease, systemic lupus erythematosus, rheumatoid arthritis, asthma, primary biliary cirrhosis, etc.[17–20,50] It has been previously shown that IKZF3 is associated with IL-10 in CD4 T cells and that IKZF3 is required in anti-cd3/CD28 monoclonal antibody-mediated IL-10 induction.[51] Knockdown of the TF IKZF3 in HER2-specific CAR T cells targeting breast cancer cells enhanced T cell activation and proliferation and significantly enhanced cancer cell killing in both in vitro and xenograft models.[52] Additionally, in MM, high expression of IKZF3 in T cells suppressed myeloma-specific T cell responses in vitro and its expression was not affected by the tumor microenvironment.[25] According to our analysis, the high expression levels of IKZF3 were significantly associated with immune cells in HNSCC, such as B cell (partial-cor = 0.411, P = 7.86e-21), $CD8^+$ T cell (partial-cor = 0.57, P = 3.35e-42), $CD4^+$ T cell (partial-cor = 0.631, P = 8.88E-55), macrophage (partial-cor = 0.475, P = 1.31e-28), neutrophil (partial-cor = 0.537, P = 3.76e-37) and dendritic cell (partial-cor = 0.649, P = 5.15e-59). Besides, our results showed the expression levels of IKZF3 were negatively correlated with tumor purity (P = 1.35e-03). It is consistent with our results shown above that high expression of IKZF3 is correlated with better OS. However, those analyses require further investigation.

There were some limitations to our study that need to be recognized. First, the sample size in our study was relatively small, and larger sample sizes are needed to increase the reliability of our findings. Second, the clinical information from the TCGA database was not comprehensive, and more clinical data concerning tumor progression and prognoses, such as smoking and drinking status should be included to better evaluate the relationship between the IKZF3 gene and HNSCC. Third, our current study based on the public databases to analyze the prognosis prediction of the expression level of the IKZF3 gene lacks verification at the protein level. Therefore, future research is still needed to address these issues. Due to the small sample size and incomplete clinical information in the current study, further well-designed and larger sample size studies are necessary to validate our results.

In summary, based on the bioinformatics analyses presented in this study, we suggested that high expression levels of IKZF3 were associated with better OS. Age and tumor grade were independent risk factors in HNSCC. We also found that IKZF3 promotes invasion and metastasis of cancer cells; suggesting that IKZF3 plays a crucial role in tumor initiation, development, and malignant behavior and may be used as a biomarker in the diagnosis and prognosis of patients with head and neck cell carcinoma. Moreover, several pathways in cancer may be pivotal pathways regulated by IKZF3 in HNSCC. Besides, IKZF3 was related to genes that observed mutation in HNSCC. We believe that IKZF3 could as a novel potential prognostic marker for HNSCC. We hope that our findings will benefit future studies and improve the prognosis of HNSCC patients.

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References

[1] Fitzmaurice C, Allen C, Barber RM, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability,

and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the Global Burden of Disease Study. JAMA Oncol. 2017;3:524–48.
[2] Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. Nat Rev Cancer. 2011;11:9–22.
[3] Vigneswaran N, Williams MD. Epidemiologic trends in head and neck cancer and aids in diagnosis. Oral Maxillofac Surg Clin North Am. 2014;26:123–41.
[4] Sankaranarayanan R, Masuyer E, Swaminathan R, et al. Head and neck cancer: a global perspective on epidemiology and prognosis. Anticancer Res. 1998;18:4779–86.
[5] Pezzuto F, Buonaguro L, Caponigro F, et al. Update on head and neck cancer: current knowledge on epidemiology, risk factors, molecular features and novel therapies. Oncology (Huntingt). 2015;89:125–36.
[6] Higgins GS, O'Cathail SM, Muschel RJ, et al. Drug radiotherapy combinations: review of previous failures and reasons for future optimism. Cancer Treat Rev. 2015;41:105–13.
[7] Fuller CD, Wang SJ, Thomas CR Jr., et al. Conditional survival in head and neck squamous cell carcinoma: results from the SEER dataset 1973-1998. Cancer. 2007;109:1331–43.
[8] Gupta S, Kong W, Peng Y, et al. Temporal trends in the incidence and survival of cancers of the upper aerodigestive tract in Ontario and the United States. Int J Cancer. 2009;125:2159–65.
[9] Joshi I, Yoshida T, Jena N, et al. Loss of Ikaros DNA-binding function confers integrin-dependent survival on pre-B cells and progression to acute lymphoblastic leukemia. Nat Immunol. 2014;15:294–304.
[10] John LB, Ward AC. The Ikaros gene family: transcriptional regulators of hematopoiesis and immunity. Mol Immunol. 2011;48:1272–8.
[11] Dittrich-Salamon M, Meyer A, Yan S, et al. Regulatory T cells from patients with rheumatoid arthritis are characterized by reduced expression of ikaros zinc finger transcription factors. Cells. 2022;11:2171.
[12] Yang L, Luo Y, Wei J. Integrative genomic analyses on Ikaros and its expression related to solid cancer prognosis. Oncol Rep. 2010;24:571–7.
[13] Yoshida T, Georgopoulos K. Ikaros fingers on lymphocyte differentiation. Int J Hematol. 2014;100:220–9.
[14] Georgopoulos K, Bigby M, Wang JH, et al. The Ikaros gene is required for the development of all lymphoid lineages. Cell. 1994;79:143–56.
[15] Oguejiofor K, Hall J, Slater C, et al. Stromal infiltration of CD8 T cells is associated with improved clinical outcome in HPV-positive oropharyngeal squamous carcinoma. Br J Cancer. 2015;113:886–93.
[16] Agrawal N, Frederick MJ, Pickering CR, et al. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. Science. 2011;333:1154–7.
[17] Stransky N, Egloff AM, Tward AD, et al. The mutational landscape of head and neck squamous cell carcinoma. Science. 2011;333:1157–60.
[18] Wang H, Xu Z, Du W, et al. N160 of aiolos determines its DNA-binding activity. Anat Rec (Hoboken). 2019;302:2014–9.
[19] Tomczak K, Czerwinska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. Contemp Oncol (Pozn). 2015;19:A68–77.
[20] Chandrashekar DS, Bashel B, Balasubramanya SAH, et al. UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia. 2017;19:649–58.
[21] Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013;6:pl1.
[22] Li T, Fan J, Wang B, et al. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res. 2017;77:e108–10.
[23] Wang J, Vasaikar S, Shi Z, et al. WebGestalt 2017: a more comprehensive, powerful, flexible and interactive gene set enrichment analysis toolkit. Nucleic Acids Res. 2017;45:W130–7.
[24] Franceschini A, Szklarczyk D, Frankild S, et al. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res. 2013;41:D808–15.
[25] Awwad MHS, Kriegsmann K, Plaumann J, et al. The prognostic and predictive value of IKZF1 and IKZF3 expression in T-cells in patients with multiple myeloma. Oncoimmunology. 2018;7:e1486356.
[26] Hung KH, Su ST, Chen CY, et al. Aiolos collaborates with Blimp-1 to regulate the survival of multiple myeloma cells. Cell Death Differ. 2016;23:1175–84.
[27] Duhamel M, Arrouss I, Merle-Beral H, et al. The Aiolos transcription factor is up-regulated in chronic lymphocytic leukemia. Blood. 2008;111:3225–8.
[28] Li L, Ding X, Wang X, et al. Polymorphisms of IKZF3 gene and autoimmune thyroid diseases: associated with graves' disease but not with Hashimoto's Thyroiditis. Cell Physiol Biochem. 2018;45:1787–96.
[29] Cai X, Qiao Y, Diao C, et al. Association between polymorphisms of the IKZF3 gene and systemic lupus erythematosus in a Chinese Han population. PLoS One. 2014;9:e108661.
[30] Lessard CJ, Adrianto I, Ice JA, et al. Identification of IRF8, TMEM39A, and IKZF3-ZPBP2 as susceptibility loci for systemic lupus erythematosus in a large-scale multiracial replication study. Am J Hum Genet. 2012;90:648–60.
[31] Kurreeman FA, Stahl EA, Okada Y, et al. Use of a multiethnic approach to identify rheumatoid- arthritis-susceptibility loci, 1p36 and 17q12. Am J Hum Genet. 2012;90:524–32.
[32] Acevedo N, Reinius LE, Greco D, et al. Risk of childhood asthma is associated with CpG-site polymorphisms, regional DNA methylation and mRNA levels at the GSDMB/ORMDL3 locus. Hum Mol Genet. 2015;24:875–90.
[33] Juran BD, Hirschfield GM, Invernizzi P, et al. Immunochip analyses identify a novel risk locus for primary biliary cirrhosis at 13q14, multiple independent associations at four established risk loci and epistasis between 1p31 and 7q32 risk variants. Hum Mol Genet. 2012;21:5209–21.
[34] Tong CC, Kao J, Sikora AG. Recognizing and reversing the immunosuppressive tumor microenvironment of head and neck cancer. Immunol Res. 2012;54:266–74.
[35] He LC, Gao FH, Xu HZ, et al. Ikaros inhibits proliferation and, through upregulation of Slug, increases metastatic ability of ovarian serous adenocarcinoma cells. Oncol Rep. 2012;28:1399–405.
[36] Sharma B, Shah R, Jamwal RS, et al. Evaluation of newly identified Ikaros family zinc finger 1 loci in colorectal cancer. J Cancer Res Ther. 2021;17:46–50.
[37] Hung JJ, Kao YS, Huang CH, et al. Overexpression of Aiolos promotes epithelial-mesenchymal transition and cancer stem cell-like properties in lung cancer cells. Sci Rep. 2019;9:2991.
[38] Edgren H, Murumagi A, Kangaspeska S, et al. Identification of fusion genes in breast cancer by paired-end RNA-sequencing. Genome Biol. 2011;12:R6.
[39] Li X, Xu Z, Du W, et al. Aiolos promotes anchorage independence by silencing p66Shc transcription in cancer cells. Cancer Cell. 2014;25:575–89.
[40] Mo BY, Li GS, Huang SN, et al. Laryngeal squamous cell carcinoma: potential molecular mechanism and prognostic signature based on immune-related genes. Med Sci Monit. 2020;26:e928185.
[41] Luczak MW, Jagodzinski PP. The role of DNA methylation in cancer development. Folia Histochem Cytobiol. 2006;44:143–54.
[42] Momparler RL. Cancer epigenetics. Oncogene. 2003;22:6479–83.
[43] Li J, Huang Q, Zeng F, et al. The prognostic value of global DNA hypomethylation in cancer: a meta-analysis. PLoS One. 2014;9:e106290.
[44] Franzen A, Vogt TJ, Muller T, et al. PD-L1 (CD274) and PD-L2 (PDCD1LG2) promoter methylation is associated with HPV infection and transcriptional repression in head and neck squamous cell carcinomas. Oncotarget. 2018;9:641–50.
[45] Basu B, Chakraborty J, Chandra A, et al. Genome-wide DNA methylation profile identified a unique set of differentially methylated immune genes in oral squamous cell carcinoma patients in India. Clin Epigenetics. 2017;9:13.
[46] Warta R, Herold-Mende C, Chaisaingmongkol J, et al. Reduced promoter methylation and increased expression of CSPG4 negatively influences survival of HNSCC patients. Int J Cancer. 2014;135:2727–34.
[47] Esteller M, Herman JG. Cancer as an epigenetic disease: DNA methylation and chromatin alterations in human tumours. J Pathol. 2002;196:1–7.
[48] Laubli H, Borsig L. Altered cell adhesion and glycosylation promote cancer immune suppression and metastasis. Front Immunol. 2019;10:2120.
[49] Marshall EA, Ng KW, Kung SH, et al. Emerging roles of T helper 17 and regulatory T cells in lung cancer progression and metastasis. Mol Cancer. 2016;15:67.
[50] Terada LS, Liu Z. Aiolos and lymphocyte mimicry in lung cancer. Mol Cell Oncol. 2014;1:e29912.
[51] Ridley ML, Fleskens V, Roberts CA, et al. IKZF3/Aiolos is associated with but not sufficient for the expression of IL-10 by CD4(+) T cells. J Immunol. 2020;204:2940–8.
[52] Zou Y, Liu B, Li L, et al. IKZF3 deficiency potentiates chimeric antigen receptor T cells targeting solid tumors. Cancer Lett. 2022;524:121–30.