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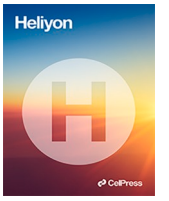

Research article

HIF-1A as a prognostic biomarker related to invasion, migration and immunosuppression of cervical cancer

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ABSTRACT

Background: The incidence of cervical cancer ranks second among malignant tumors in women, exerting a significant impact on their quality of life and overall well-being. The hypoxic microenvironment plays a pivotal role in the initiation and progression of tumorigenesis. The present study aims to investigate the fundamental genes and pathways associated with the hypoxia-inducible factor (HIF-1A) in cervical cancer, aiming to identify potential downstream targets for diagnostic and therapeutic purposes.

Methods: We obtained dataset GSE63514 from the Comprehensive Gene Expression Database (GEO). The dataset comprised of 24 patients in the normal group and 28 patients in the tumor group. Gene set difference analysis (GSVA) and gene set enrichment analysis (GSEA) were used to identify the genes related to HIF-1A expression and the specific signaling pathways involved.

The association between HIF-1A and tumor immune infiltration was examined in the TCGA dataset. The WGCAN network was constructed to identify key genes within the blue module, and subsequent gene ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted to determine the pathways and functional annotations associated with HIF-1A. The protein interaction network of the HIF-1A gene was obtained from the STRING database and visualized using Cytoscape in the meantime.

The function of HIF-1A and its related gene expression were verified in vivo.

Results: HIF-1A was a risk factor in both univariate and multivariate Cox regression analysis of cervical cancer patients. A total of 344 genes significantly correlated with the expression of HIF-1A were identified through correlation analysis, and the genes exhibiting the strongest correlation were obtained. The major signaling pathways involved in HIF-1A encompass TNF-α/NF-κB, PI3K/AKT/MTOR, TGF-β, JAK-STAT, and various other signaling cascades. Reinforced by qRT-PCR, we identified Integrin beta-1 (ITGB1), C–C motif chemokine ligand 2 (CCL2), striatin 3 (STRN3), and endothelin-1 (EDN1) as pivotal downstream genes influenced by HIF-1A. HIF-1A is associated with immune infiltration of natural killer (NK) cells, mast cells, $CD4^{+}$T cells, M0 macrophages, neutrophils, follicular helper T cells, $CD8^{+}$T cells, and regulatory T cells (Treg). HIF-1A is associated with sensitivity to chemotherapy drugs. The identification of the HIF-1A pathway and its function primarily focuses on cytoplasmic translation, aerobic respiration, cellular respiration, oxidative phosphorylation, thermogenesis, among others. The results of in vivo experiments have confirmed that HIF-1A plays a crucial role in promoting the migration and invasion of cervical cancer cells. Moreover, the overexpression of HIF-1A led to an upregulation in the expressions of ITGB1, CCL2, STRN3, and EDN1.

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Conclusions: The role of HIF-1A in cervical cancer was determined through a combination of bioinformatics analysis and experimental validation. The genes potentially implicated in the tumorigenesis mechanism of HIF-1A were identified. These findings has the potential to enhance our comprehension of the progression of cervical cancer and offer promising therapeutic targets for its clinical management.

1. Introduction

Cervical cancer is a global health problem with approximately 600,000 cases and 340,000 deaths each year. Cervical cancer was the fourth leading cause of cancer deaths in 2018, accounting for 8 % of cancer deaths among women worldwide, The average age of cervical cancer diagnosis was 53 years and the average age of death was 59 years [1–3]. Cervical cancer presents some unique features that differ from other solid tumors. Healthy lamellar epithelium, including cervical cells, has the characteristics of chronic hypoxic tissue while levels of HIF-1A are significantly increased in advanced cervical cancer [4]. Patients in the early stage of surgery can be effectively treated, while the efficacy is limited for patients in the middle and late stages, and the combination of chemoradiotherapy will produce additional toxic damage. In addition, recurrent or metastatic cervical cancer is often difficult to treat and has a poor prognosis [5]. This indicates the need for a deeper understanding of the pathogenesis of cervical cancer and the exploration of new therapeutic modalities such as targeted therapy and immunotherapy for these patients.

The growth microenvironment of solid tumors is commonly believed to be characterized by an oxygen-deprived state. HIF-1A plays a pivotal role in tumor development [6]. The expression of HIF-1A is significantly upregulated in various tumors, which is intricately associated with cellular metastasis, angiogenesis and treatment [7–9]. The accumulation of HIF-1A in the nucleus can also regulate downstream genes through various mechanisms to promote tumor cell proliferation, invasion, energy metabolism, epithelial-mesenchymal transformation (EMT), immune escape, etc, leading to tumor metastasis and poor prognosis [10–12]. The findings suggest that targeting the molecular pathways associated with HIF-1A could potentially serve as a therapeutic approach for cervical cancer.

In this study, the mRNA expression data of 52 patients with cervical cancer obtained from GEO and TCGA databases were analyzed by gene differential expression analysis and WGCNA analysis, and the expression level of HIF-1A in cervical cancer and its relationship with the prognosis of cervical cancer were comprehensively analyzed. To further understand its role in the development of cervical cancer, GSVA and GSEA were also used to explore potential mechanisms, and 10 HUB genes with positive/negative correlation coefficients were obtained. The functional annotation and signal pathway analysis of its related genes were carried out by WGCAN network. The proteins interacting with HIF-1A were discovered through the STRING database. We also investigated the association between HIF-1A expression and the level of immune invasion, drug sensitivity, and tumor mutation load (TMB) of cervical cancer.

This study found that the overexpression of HIF-1A can promote the migration and invasion of cervical cancer cells, which may play a role through ITGB1. According to these findings, HIF-1A/ITGB1 may provide a target for the treatment of cervical cancer.

2. Materials and methods

2.1. Data acquisition and difference analysis

TCGA database (https://portal.gdc.cancer.gov/) as the biggest cancer gene information database, including gene expression data, such as copy number variation, SNP data. We downloaded the original mRNA expression data and SNP data of cervical cancer data for subsequent analysis. A total of 309 specimens were collected. The Series Matrix File data file of GSE63514 related to cervical cancer was downloaded from the NCBI GEO public database, and the annotation file was GPL570. Expression profile data of 52 patients were included, including 24 cases in the normal group and 28 cases in the tumor group, to analyze the difference in HIF1A expression.

2.2. Co-expression analysis

The co-expression of HIF-1A gene in cervical cancer data was analyzed. The filtering condition of correlation coefficient was 0.4, and the p value was 0.01. After screening the genes with the most significant expression of HIF-1A, the HIF1A correlation analysis circle and heat map were drawn with "corrplot" and "circlize" packages.

2.3. Analysis of immune cell infiltration

The CIBERSORT method is extensively employed for the assessment of immune cell populations within the microenvironment. Based on the principle of support vector regression, the expression matrix of immune cell subtypes was deconvolution analyzed. It contains 547 biomarkers that distinguish 22 human immune cell phenotypes, including T cells, B cells, plasma cells, and myeloid cell subsets. CIBERSORT algorithm was used to analyze the RNA-seq data of cervical cancer patients, which was used to infer the relative proportion of 22 kinds of immune infiltrating cells, and to analyze the correlation between gene expression and immune cell content. $P < 0.05$ was considered statistically significant.

2.4. Analysis of gene set differences

The GSVA is a nonparametric and unsupervised approach used to assess the enrichment of transcriptome gene sets. By comprehensively scoring the gene set of interest, GSVA converts the gene level change into pathway level change, and then judges the biological function of the sample. In this study, gene sets were downloaded from Molecular signatures database, and GSVA algorithm was used to conduct comprehensive scores for each gene set to evaluate potential biological functional changes in different samples.

2.5. GSEA analysis

According to the expression level of HIF-1A, patients were divided into high and low expression groups, and the difference of signaling pathway between high and low expression groups was further analyzed by GSEA. Background gene sets were annotated gene sets of version 7.0 downloaded from MsigDB database as annotation gene sets of subtype pathways. Differential expression analysis of pathways between subtypes was performed, and significantly enriched gene sets (adjusted p value less than 0.05) were sequenced according to consistency scores. GSEA analysis is often used to explore the close combination of disease classification and biological significance.

2.6. Drug sensitivity analysis

Genomics database based on the largest drug (GDSC cancer drugs sensitivity genomics database, https://www.cancerrxgene.org/), we use R software package "pRRophetic" to predict chemotherapy sensitivity of each tumor samples; The IC50 estimates of each specific chemotherapeutic drug were obtained by regression method, and the regression and prediction accuracy were checked by 10 cross-validations with the GDSC training set. Default values were selected for all parameters, including "combat" to remove batch effects and taking an average of repeated gene expression.

2.7. Nomogram model construction

Based on regression analysis, the Nomogram is drawn on the same plane with scaled line segments according to the expression of genes and clinical symptoms to express the relationship between variables in the prediction model. By building a multi-factor regression model, each value level of each influence factor is assigned a score according to the contribution degree of each influence factor in the model to the outcome variable (the size of the regression coefficient), and then the total score is added to obtain the predicted value.

2.8. TMB, MSI, NEO data analysis

TMB is defined as the total number of somatic gene coding errors, base substitutions, insertions, or deletions detected per million bases. The TMB in this study was defined by quantifying the frequency of variations and the variance/exon length for each tumor sample, achieved through dividing the number of non-synonymous mutation sites by the total length of the protein coding region. The MSI values for each TCGA patient were derived from previously published studies [13]. NetMHCpan v3.0 was used to evaluate tumor neoplasm antigens in each patient [14].

2.9. WGCNA analysis

By constructing the weighted gene co-expression network, the gene modules of co-expression were searched, and the correlation between gene network and HIF-1A, as well as the key genes in the network were explored. The coexpression network of all genes in cervical cancer data set was constructed using WGCNA-R package, and the genes with the top 5000 variogram were screened by this algorithm for further analysis. The soft threshold was set to 3. The weighted adjacency matrix is transformed into topological overlap matrix (TOM) to estimate the network connectivity, and the hierarchical clustering method is used to construct the cluster tree structure of TOM matrix. Different branches of the cluster tree represent different gene modules, and different colors represent different modules. Based on the weighted correlation coefficient of genes, genes were classified according to their expression patterns. Genes with similar patterns were grouped into one module, and all genes were divided into multiple modules according to gene expression patterns.

2.10. Gene module function enrichment analysis

In order to obtain the biological functions and signaling pathways involved in the key modules of WGCNA (blue module in this study, which has the highest correlation with HIF-1A), the R package "ClusterProfiler" was used for functional annotation of the module genes to comprehensively explore the functional correlation of these module genes.GO and KEGG were used to assess the relevant functional categories. GO and KEGG enrichment pathways with p and q values less than 0.05 were considered significant.

2.11. Cell culture

Human cervical cancer cells (Hela, Caski) were purchased from Procell (Wuhan, China). Hela and Caski cells were cultured using DMEM medium (Gibco) and 1640 medium (Gibco) containing 10 % fetal bovine serum (FBS, Procell), respectively. The cells were incubated in a standard incubator containing 5%CO2 at 37 °C under normal oxygen. The cells were kept at 1%O2, 5%CO2 and 94%N2 under hypoxia.

2.12. RNA extraction and quantitative RT-PCR analysis

Total RNA was extracted from cells with TRIzol reagent (Vazyme). The cDNA was synthesized by reverse transcribing 1000 ng of total RNA using the HiScript®II 1st Strand cDNA Synthesis Kit (Vazyme). The relative mRNA expression was calculated by 2−ΔΔCt. SYBR Green PCR Kit (Vazyme) was used to quantify target gene expression, and ACTB was used as internal control. All qPCR primer

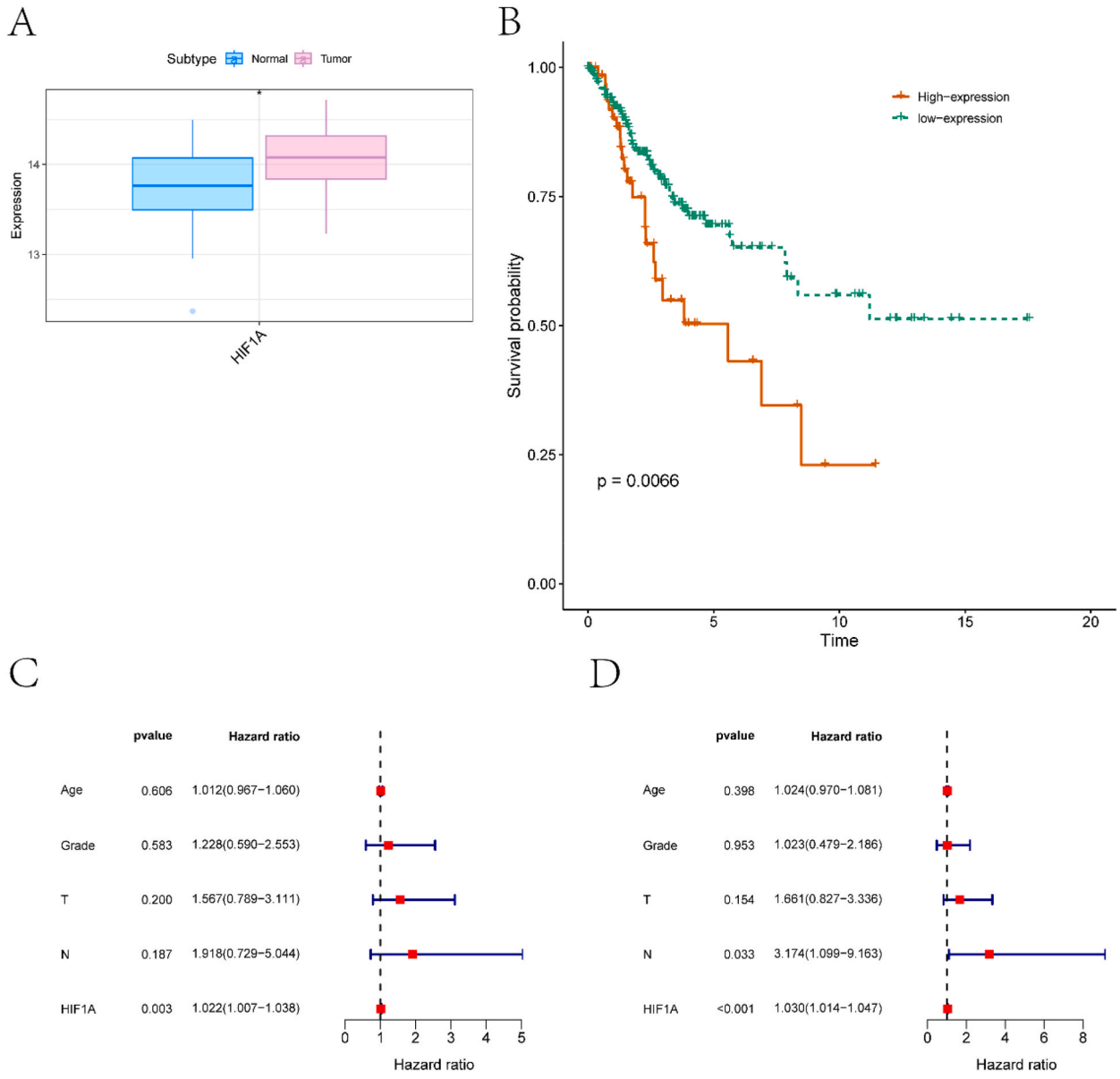

Fig. 1. Differential analysis of HIF-1A gene survival analysis and single and multiple factors. (A)Differences in HIF-1A gene expression, with blue representing control patients and pink representing disease patients. (B)KM curve of HIF-1A, with orange indicating high expression and green indicating low expression. (C–D) HIF-1A in cervical cancer patients was a risk factor in univariate and multivariate Cox regression analysis, and there was a statistical difference.

sequences are as follows:

Kinectin 1(KTN1): 5′- AAATGTCTTCGTAGATGAACCCC-3′ (forward)
5′-TTTGTCAGTTTCGGTCTTCAGTT-3′(reverse)
IGTB1: 5′-CCTACTTCTGCACGATGTGATG-3′ (forward)
5′-CCTTTGCTACGGTTGGTTACATT-3′(reverse)
CCL2: 5′-CAGCCAGATGCAATCAATGCC-3′ (forward)
5′-TGGAATCCTGAACCCACTTCT-3′(reverse)
STRN3: 5′-AGTGGGCTCGGTTCGAGAT -3′ (forward)
5′-CTTGACCTTTTCTTTCGCCTTG-3′(reverse)
EDN1 : 5′- AGAGTGTGTCTACTTCTGCCA-3′ (forward)
5′-CTTCCAAGTCCATACGGAACAA-3′(reverse)
ACTB: 5′-CATGTACGTTGCTATCCAGGC-3′ (forward)
5′-CTCCTTAATGTCACGCACGAT-3′(reverse)

2.13. Protein imprinting analysis

Cell proteins were extracted using dissolved buffers. Boil the protein in a metal bath for 10 min to denature it. Protein samples were isolated using SDS-PAGE (Beyotime Biotechnology) and then transferred to PVDF membrane (Millipore). Soak in 5 % skim milk for 1 h. Refrigerate the film at 4 °C overnight with the primary antibody (diluted to a concentration of 1:2000), followed by incubation at room temperature for 1 h with the secondary antibody (diluted to a concentration of 1:5000). The strips were exposed to ECL luminescent solution (Thermo). Antibodies to HIF-1A, E-cadherin, Vimentin, Snail, β-actin, ITGB1 were purchased from Proteintech (China).

2.14. Transwell experiment

The matrix glue (CORNING) was diluted at 1:8 with FBS-free DMEM and 1640, and the Transwell chamber with a pore size of 8 μm was covered with the matrix glue and then placed in a 24-well plate. The Transwell cell culture system coated with matrix gel was employed for invasion assays, whereas the uncoated Transwell inserts were utilized for migration assays. Cells with different treatments (6 × 104 for migration and 1 × 105 for invasion) were seeded in Transwell chambers. The upper chamber was supplemented with 200 μL of FBS-free medium, while the lower chamber was supplemented with 600 μL of 20 % FBS medium. After 24 h, the chamber was removed, the cells were fixed in methanol for 20 min, and the cells were stained with 1 % crystal violet for 20 min. The upper chamber cells were wiped clean with a cotton swab, counted and photographed.

2.15. Wound healing test

In the 24-well plate, when the density reached 100 %, the cells were vertically scraped with the tip of a 10ul pipette, washed with pre-cooled PBS for 3 times, and then added to the serum-free medium. The photos were taken at 0:00 and 24:00 respectively.

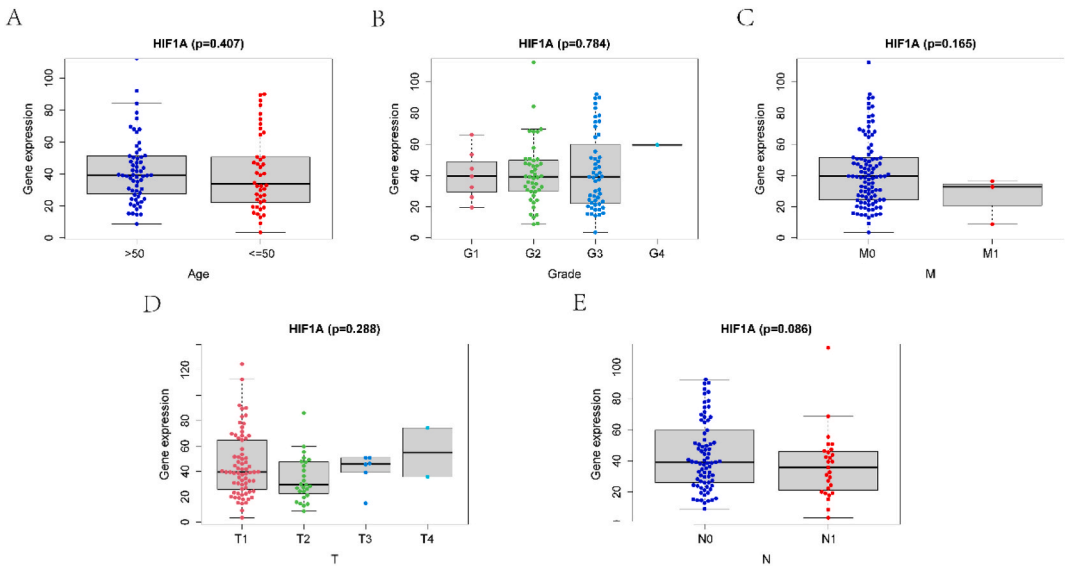

Fig. 2. Relationship between HIF-1A and clinical symptoms. (A–E) Clinical correlation analysis of HIF-1A gene in cervical cancer data. Clinical correlation analysis mainly analyzed the survival time, survival state and tumor TNM of the patients.

2.16. Statistical analysis

The database analysis was carried out in R language (version 4.3.0), $p < 0.05$ was statistically significant. The experimental data were analyzed by GraphPad Prism 9.5 statistical software. At least three experimental data were analyzed using *t*-test and their differences were analyzed(*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$).

3. Results

3.1. HIF-1A expression prognosis and clinical relevance

The specific mechanism of HIF-1A in cervical cancer is unknown. We downloaded cervical cancer-related GSE63514 expression data from the GEO database and showed that HIF-1A expression was significantly different between normal and cancerous tissues (Fig. 1A). Our survival analysis based on the high and low expression levels of HIF-1A gene in the TCGA database showed that HIF-1A was significant when the optimal cut-off value was taken (Fig. 1B). Based on clinical data and HIF-1A gene expression level, univariate and multivariate Cox regression models were established and forest maps were drawn. The results showed that HIF-1A was a risk factor in patients with cervical cancer in univariate and multivariate Cox regression analysis, with statistical difference (Fig.. 1C–.D). In addition, we analyzed the relationship between HIF-1A and clinical symptoms (Fig .. 2A–E).

3.2. HIF-1A co-expression network and immunoinfiltration and enrichment analysis

We further explored the co-expression network of HIF-1A by correlation analysis according to the expression profile of cervical cancer patients in TCGA database. The filtering condition of correlation coefficient was 0.4, and the *p* value was 0.01. A total of 344 genes were screened out that were significantly correlated with HIF-1A expression. The heat map of the TOP10 genes with positive/negative correlation coefficients (Fig. 3A) and the coexpression correlation circle diagram (Fig. 3B) were shown. The tumor microenvironment is mainly composed of tumor-related fibroblasts, immune cells, extracellular matrix, various growth factors, inflammatory factors, special physicochemical characteristics and cancer cells themselves, etc. The tumor microenvironment significantly affects the diagnosis, survival outcome and clinical treatment sensitivity of tumors. By analyzing the relationship between HIF-1A and tumor immune invasion in TCGA dataset, the potential molecular mechanism of HIF-1A affecting cervical cancer progression was further explored. The results showed that HIF-1A was positively correlated with NK cells resting, Mast cells activated, Macrophages M0, T cells CD4 memory resting and Neutrophils. It was significantly negatively correlated with T cells follicular helper, NK cells

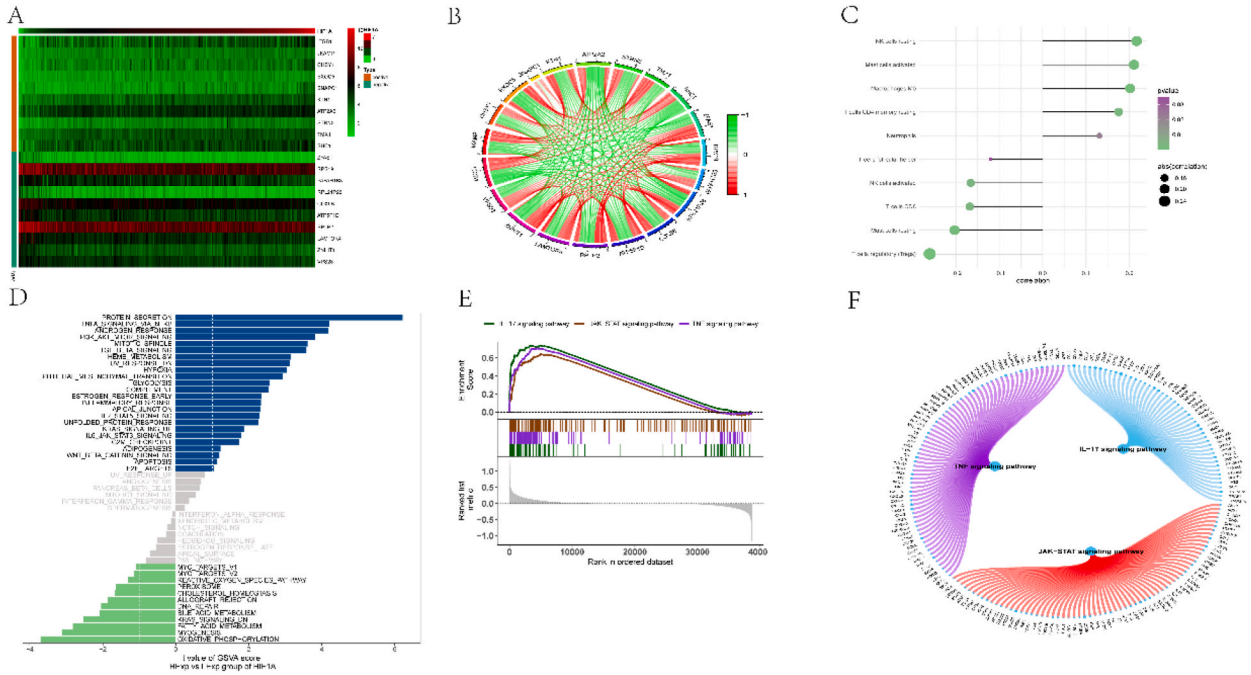

Fig. 3. HIF-1A correlation analysis and gene-related pathway analysis (A–B) Correlation coefficient positive/negative correlation of TOP10 genes and co-expression correlation, red positive correlation, green negative correlation. (C) Correlation between gene expression and immune cell content. (D) HIF-1A gene-related GSVA analysis. Pathway activity of key genes in the high expression group and the low expression group was scored by GSVA to explore the pathway differences between the high risk group and the low risk group. The background gene set was Hallmark. (E–F) KEGG signaling pathway for GSEA analysis of HIF-1A gene, as well as pathway regulation and involved genes.

activated, T cells CD8, Mast cells resting and T cells regulatory (Fig. 3C). Next, we will investigate the specific signaling pathway involved in HIF-1A gene and explore the potential molecular mechanism of HIF-1A gene's influence on tumor progression. The GSVA results revealed that patients exhibiting high expression of HIF-1A were significantly enriched in TNFA_SIGNALING_VIA_NFKB, PI3K_AKT_MTOR_SIGNALING, TGF_BETA_SIGNALING, and other crucial signaling pathways (Fig. 3D). In addition, GSEA results showed that HIF-1A could enrich IL-17 signaling pathway, JAK-STAT signaling pathway, and TNF signaling pathway (Fig .. 3E–F). It is suggested that HIF-1A may affect cervical cancer progression through these pathways.

3.3. Relationship between drug sensitivity and immunotherapy markers of HIF-1A

We downloaded the processed SNP related data of cervical cancer, selected TOP30 genes with high mutation frequency as display, compared the differences of mutant genes between the two groups of patients, and drew the mutation landscape map with R ComplexHeatmap (Fig. 4A) , The results showed the proportion of HIF-1A related gene mutations as shown in the figure. The treatment effect of surgery combined with chemotherapy for early cervical cancer is clear. Based on the drug sensitivity data of GDSC database, our study used R packet "pRRophetic" to predict the chemotherapy sensitivity of each tumor sample, and further explored the sensitivity of HIF1A and common chemotherapy drugs. The results showed that HIF-1A was associated with the sensitivity of ABT.263, ABT.888, AMG.706, AUY922, Bexarotene, Bicalutamide (Fig. 4B). We further explored the relationship between HIF-1A gene and common immunotreatment-related tumor markers, and found that there were significant differences between the high-low expression group of HIF-1A gene and tumor Neoantigen and tumor mutation load (TMB) (Fig .. 4C–E).

3.4. The construction of the network is co-expressed by the nomogram and WGCNA

The results of their regression analysis were presented in the form of a histogram by HIF-1A expression. The results of regression analysis showed that in all our samples, the values of different clinical indicators of cervical cancer and the distribution of HIF-1A expression contributed to different degrees in the whole scoring process (Fig. 5A). At the same time, we also conducted a prediction analysis (Fig. 5B) of the OS in one year and three years, and the results showed that the predicted OS was in good agreement with the observed OS, and the Nomogram model had a good prediction performance. We further constructed WGCNA network based on cervical cancer data to explore the regulatory network related to HIF-1A in cervical cancer. Soft threshold β was set to 3 (Fig. 5C), and then gene modules were detected based on tom matrix. A total of 12 gene modules were detected in cervical cancer. They were brown (423), blue(2468), cyan(72), green (298), salmon (76), tan (87), black (644), magenta (142), pink (238), midnightblue (68), and green yellow (116), yellow (368). Through further analysis between modules and traits, we found that blue module had the highest correlation with HIF-1A (cor = −0.36, p = 4e-11) (Fig .. 5D–E). We further used the blue module gene for pathway analysis. GO results

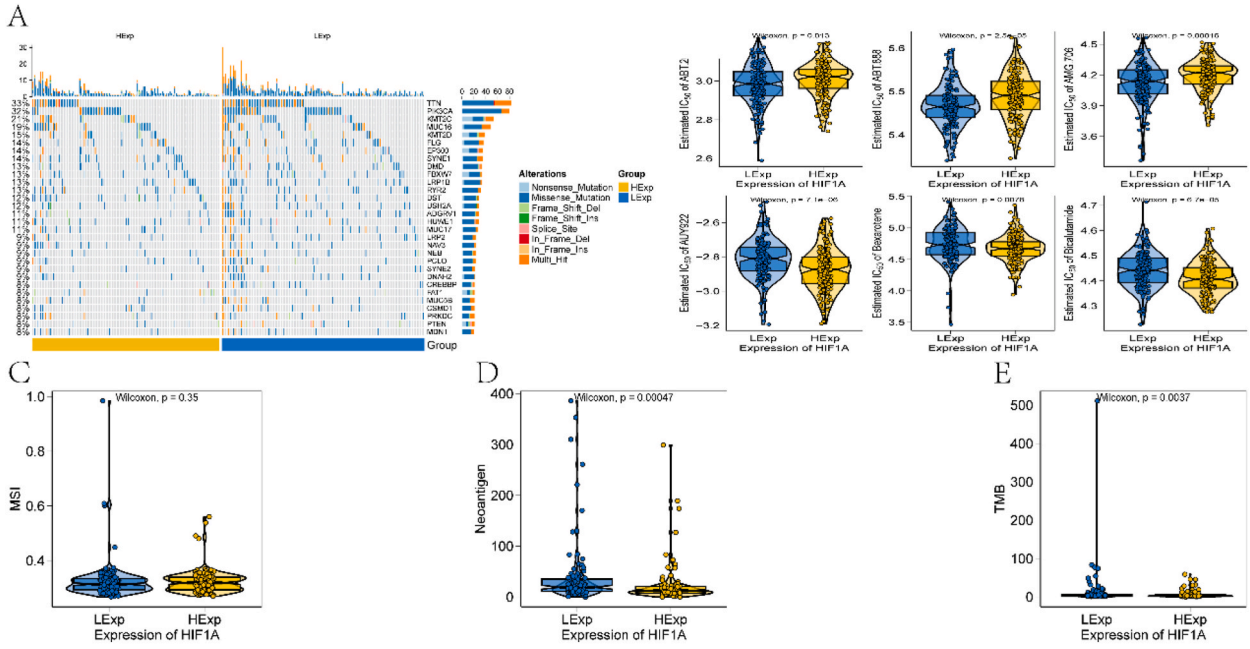

Fig. 4. Cervical cancer related gene mutations and sensitivity to common chemotherapy drugs. (A) SNP-related data of cervical cancer. TOP30 genes with high mutation frequency were selected as display, and the differences of mutated genes between the two groups were compared. (B) Based on the drug sensitivity data from the GDSC database, the potential therapeutic agents predicted were ABT.263, ABT.888, AMG.706, AUY922, Bexarotene, Bicalutamide. (C–E) To investigate the differences between HIF-1A gene expression and tumor microsatellite instability (MSI), Neoantigen, and tumor mutation load (TMB).

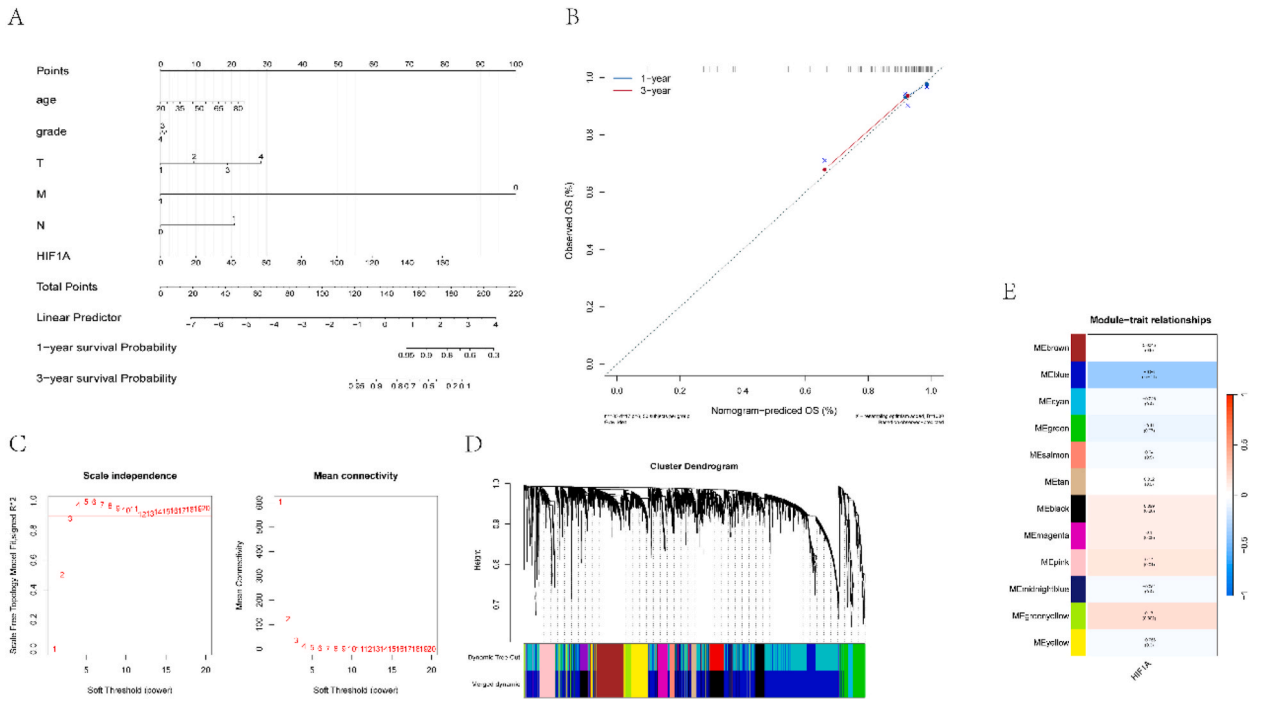

Fig. 5. Nomogram model construction and WGCNA analysis (A) The values of different clinical indicators of cervical cancer and the distribution of HIF-1A expression contributed to different degrees in the whole scoring process. (B) Forecast analysis of OS conditions for one - and three-year periods. (C) Scale-free index and average connectivity of each soft threshold for cervical cancer. (D) A tree of cervical cancer gene clusters, with different colors representing different modules. (E) Heat map of the correlation between the module signature gene and HIF-1A, with negative correlation in blue and positive correlation in red.

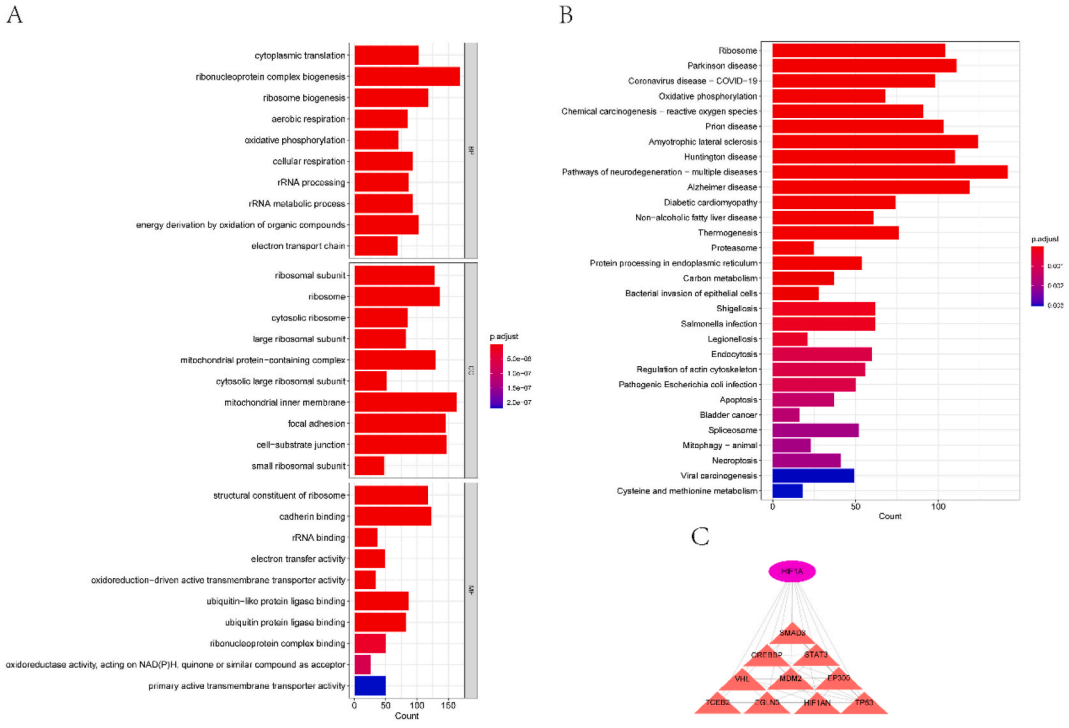

Fig. 6. Gene enrichment analysis (A–B) ClusterProfiler-based GO-KEGG enrichment analysis. (C) HIF-1A's protein interaction network, based on String database, visualized by Cytoscape software.

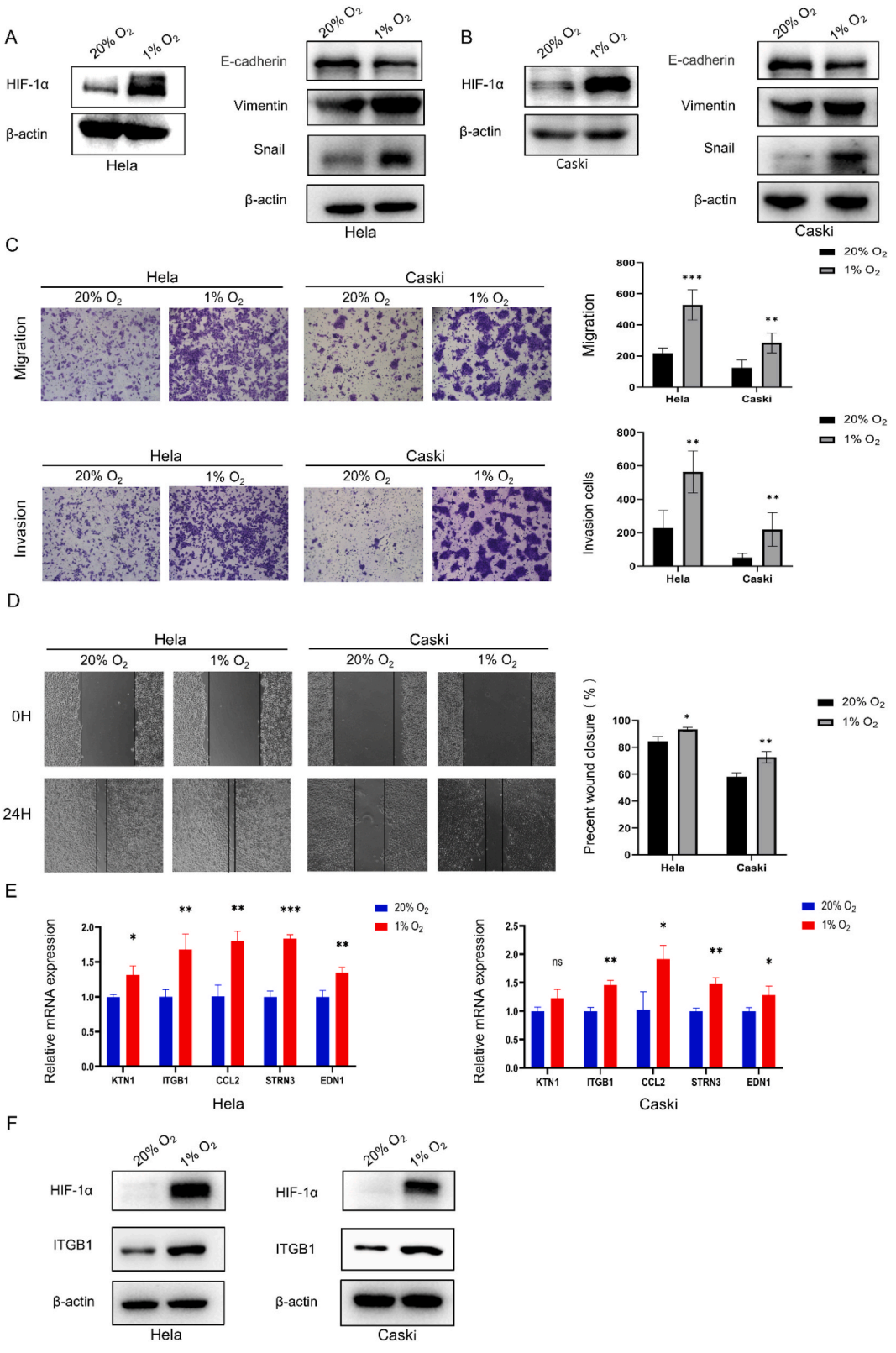

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Fig. 7. Hypoxia promotes cervical cancer cell migration and invasion and downstream gene expression through HIF-1A (A–B) Western blot analysis was used to determine the protein levels of HIF-1A and EMT markers in Hela and Caski cells in anoxic environment (C) Transwell migration and invasion tests and (D) wound scratch tests to examine the migration and invasion capacity of Hela and Caski cells in anoxic environments (E) The expressions of KTN1, ITGB1, CCL2, STRN3 and EDN1 in anoxic environment were detected by qPCR (F) Western blot analysis to detect the effects of hypoxia on HIF-1A and ITGB1 *P < 0.05, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

showed that the gene was mainly enriched in cytoplasmic translation, aerobic respiration, cellular respiration and other pathways (Fig. 6A). KEGG results showed that the genes were mainly enriched Oxidative phosphorylation, Thermogenesis and other pathways (Fig. 6B). Meanwhile, the protein interaction network of HIF-1A gene was obtained using STRING database and visualized using Cytoscape (Fig. 6C).

3.5. Highly expressed HIF-1A promotes the migration and invasion of cervical cancer cells

Next, we examined the migratory and invasive potential of cervical cancer cells under 1 % hypoxic conditions. HIF-1A was highly expressed in Hela and Caski cells in hypoxic environment. The overexpression of HIF-1A led to a downregulation in the expression of EMT-related protein E-cadherin, while there was an upregulation in the expression of Vimentin and Snail (Fig. 7A–B). The Transwell migration and invasion assay reflected the effect of HIF-1A on cell invasion (Fig. 7C). The wound healing experiment demonstrated a significant increase in the rate of cell regeneration at 24 h following HIF-1A overexpression (Fig. 7D). The results showed that HIF-1A overexpression could promote cell migration and invasion.

3.6. Hypoxia induced high expression of ITGB1

Based on the results of bioinformatics analysis, we selected KTN1, ITGB1, CCL2, STRN3 and EDN1 as downstream genes affected by HIF-1A. The mRNA expression of these genes of Hela and Caski was detected by qRT-PCR, which showed that the expressions of ITGB1, CCL2, STRN3 and EDN1 were increased in anoxic environment (Fig. 7E). The protein imprinting analysis further selected ITGB1, which exhibited significant expression when co-expressed with HIF-1A (Fig. 7F).

4. Discuss

The presence of hypoxia is a prevalent characteristic observed in solid tumors and serves as an unfavorable prognostic factor for various types of cancers, including prostate cancer, breast cancer, and cervical cancer [15]. The hypoxia response is mainly mediated by HIF-1A. Our results show that HIF-1A has a close clinical relationship with cervical cancer patients, showing a positive correlation in age, tumor stage and other aspects, and the survival time is relatively shortened with the increase of HIF-1A expression. The poor prognosis of patients with HIF-1A induced tumor or tumor progression is related to many aspects. Oxygen tension value < 10 mmHg is associated with increased mortality and metastasis from head and neck cancer, breast cancer, and cervical cancer [16]. The process of EMT can be facilitated by hypoxia through the activation of HIF-1A, which can be accomplished via various signaling pathways [17]. Analysis of GSVA and GSEA results indicated that hypoxia activated HIF-1A could affect cervical cancer progression through multiple signaling pathways. The signaling pathways involved primarily encompass TNF-α/NF-κB, PI3K/AKT/MTOR, TGF-β, JAK-STAT, and other cascades. These pathways play an important role in tumor from the aspects of inflammation, immunity, EMT, metabolism, etc [17,18].

Solid tumors produce ATP through glycolysis and lactic fermentation even under normal oxygen conditions, an effect known as the Warburg effect. Several key enzymes in glycolysis are induced by HIF-1A to activate the glycolysis pathway [19]. The increase of HIF-1A regulates the level of lactic acid, and high levels of lactic acid are associated with high tumor aggressiveness, high metastasis rate, high mortality and drug resistance. Lactic acid, in turn, stabilizes HIF-1A in cells [20]. Glutamine and serine are key amino acids for cancer cell growth, and their expression and utilization increase under HIF-1A induction [21]. HIF-1A activation can also regulate fat metabolism, thereby promoting cancer progression and leading to poor prognosis [22].

The infiltration of immune cells is significantly related to the pathogenesis of cervical cancer [23]. By analyzing the TCGA dataset, we identified the infiltration of follicular helper T cells, natural killer cells, $CD8^+$T cells, mast cells, and Tregs associated with HIF-1A in cervical cancer. Hypoxia can hinder the development and proliferation of $CD8^+$T cells in the tumor microenvironment [24]. Under hypoxic conditions, the upregulation of HIF-1A results in a reduction in pyruvate to acetyl-CoA conversion, hindering glucose entry into the tricarboxylic acid (TCA) cycle, promoting lactic acid production, and further compromising T cell anti-tumor functionality [25,26]. Hypoxia not only limits the infiltration of $CD8^+$T cells into TME, but also drives the terminal differentiation of $CD8^+$T cells, resulting in a worse prognosis for cancer patients [27]. NK cells play a key role in the immune response to infection and tumor growth, and high levels of tumor NK cell infiltration are associated with a good prognosis for cancer [28]. Enhanced transcription factor HIF-1A can inhibit NK cell activity [29]. In addition, TME acidification caused by lactic acid induced by hypoxia can induce NK cell apoptosis [28]. Tregs are immunosuppressive $CD4^+$T cells that are used to maintain immune tolerance and tissue homeostasis, and to prevent autoimmunity. Low oxygen in TME can affect the function and stability of TREGs [30]. Hypoxia may induce overexpression of CCL28 and IL-23 through HIF-1A, and then recruit Tregs to inhibit T cell function and cause tumor immune escape [12]. The excessive activity of HIF-1A has been observed to impede the function of Tregs and facilitate the onset of inflammation [31]. Follicular T helper cells are also a subpopulation of $CD4^+$T cells that contribute to immunoglobulin production and the development of memory B cells and

long-lived plasma cells [32]. HIF1A -dependent glycolysis and oxidative phosphorylation are important for germinal center reactions and follicular T-cell differentiation, and play critical roles in immunity [33]. HIF-1A induces increased expression of CCL2 and VEGF. These two proteins can recruit mast cells to reach the tumor area, and then mast cells differentiate into M1 or M2 types, in which M2 plays a role in promoting tumor development [34]. At the same time, the presence of mast cells is associated with higher Treg cell infiltration, and the mediators secreted by mast cells can induce the migration of Tregs cells to the tumor and promote immunosuppression [35]. These results further demonstrate the important role of HIF-1A induced immune infiltration in cervical cancer progression.

Moreover, it is imperative to identify novel downstream targets of HIF-1A in order to prevent the recurrence and progression of cervical cancer following hypoxia. Through bioinformatics analysis, genes significantly related to HIF-1A expression were screened out. Combined with qRT-PCR detection results, ITGB1, CCL2, STRN3 and EDN1 were selected as important downstream genes affected by HIF-1A.Chemokine CCL2 plays an important role in inflammation and cancer cell migration and invasion [36]. CCL2 is involved in tumor immune escape and angiogenesis, thereby causing tumor cell metastasis [37]. The previous studies have demonstrated that chronic hypoxia induces a down-regulation of CCL2 expression in ovarian cancer and HUVEC, while significantly up-regulating CCL2 expression in gastric cancer, cervical cancer, breast cancer cells, and dermal fibroblasts [38,39]. Hippo signaling pathway plays a key role in biological ontogeny, especially in the process of tissue and organ size and homeostasis regulation, and plays a very important role in tumorigenesis and immune response. The disorder of Hippo signaling pathway will lead to the occurrence, migration and invasion of tumor cells. Hypoxia conditions promote the activity of oncoprotein in Hippo signaling pathway [40]. STRN3 can inhibit Hippo pathway, decrease YAP phosphorylation level and increase YAP activity, and further promote the expression of cancer-related genes and promote tumorigenesis [41]. Under hypoxic conditions, β-Arrestin can interact with HIF-1A to enhance downstream expression and release of vascular EDN1 and vascular endothelial growth factor (VEGF), thereby promoting angiogenesis, tumor cell survival, and invasion. Endothelin-1 can also activate tumor cell angiogenesis, survival, epithelial-mesenchymal transformation, and metastasis through endotoxin receptor A [42]. According to the results of TCGA expression profile, qRT-PCR and protein imprinting analysis, we will focus on ITGB1 (integrin β-1) gene. The integrins, as cell membrane receptors, mediate apoptosis, cell adhesion, proliferation and migration by modulating gene expression through binding to the extracellular matrix. They play a pivotal role in cellular growth, immunosuppression and tumor progression [43]. ITGB1 was positively correlated with TGF-β and WNT signaling pathways in both breast and gastric cancer studies [44,45]. ITGB1 can induce EMT by activating TGF-β/WNT [46]. The overexpression of ITGB1 is significantly associated with the invasion rate of tumor-associated macrophages (TAM) and Treg cells in pancreatic cancer, potentially leading to immunosuppression within the context of pancreatic cancer [47]. The study on breast cancer revealed that ITGB1 is associated with lymphovascular development and lymphangiogenesis in both physiological and pathological conditions [48]. There is limited research on the direct impact of HIF-1A on ITGB1; however, it is evident that HIF-1A exerts its influence on epigenetic inheritance by modulating downstream genes to regulate ITGB1 as an upstream transcription factor. HIF-1A has the ability to bind to sucrose non-fermentation-1-associated protein kinase (SNRK), leading to an up-regulation of SNRK expression. Subsequently, it forms a complex with SP1 and binds to the ITGB1 promoter, thereby promoting endothelial cell migration and angiogenesis [49]. Other studies have shown that hypoxia-induced HIF-1A activates the m6A demethylase ALKBH5, which regulates the expression of ITGB1 in a M6A-YthDF2-dependent manner and promotes the phosphorylation of adhesion kinase (FAK), thus promoting lymphangiogenesis and metastasis of ovarian cancer [50]. In a study of the molecular network regulating hypoxia in gliomas, HIF-1A binds to the CEBPD promoter and activates ITGB1 and FN1, thereby promoting tumor invasion through the EGFR/PI3K/AKT pathway [51]. In clinical terms, ITGB1 facilitates tumor resistance to a range of anti-cancer drugs, including erlotinib, bevacizumab, gemcitabine, and gefitinib [52–55]. The impact of ITGB1 on radiation resistance may be attributed to its influence on DNA repair mechanisms and the promotion of YAP1-induced epithelial-mesenchymal transition [56]. One study found that HIF-1A and ITGB1 are associated with tumor hardness and can be used as novel predictive markers of pathological response to neoadjuvant chemotherapy [57].

Many drugs that inhibit the HIF-1A pathway have shown significant anticancer activity in clinical trials. The main regulatory mechanisms of HIF-1A include: inhibition of HIF-1A mRNA or protein expression; Promote HIF-1A protein degradation; Inhibition of HIF-1A and HIF-1β dimerization; Inhibit HIF-1A binding to DNA [58]. MO-2097 and its derivatives act on HIF-1A to inhibit tumor cells and induce tumor cell apoptosis [59]; Acriflavin (ACF) can prevent HIF-1A/β dimerization, and large dose repeated injection can inhibit tumor to a certain extent [60]; Topotecan treats ovarian cancer by inhibiting hypoxia-induced HIF-1A protein and DNA-binding activity [61]. A variety of HIF-1A inhibitors can play a role in different stages, providing more options for combination with other chemotherapy agents and surgical protocols, and providing more ideas for the treatment of solid tumors.

This experiment has some limitations. HIF-1A can increase the cycle and apoptosis test. In future experiments, functional experiments of downstream genes will be added and the combination relationship between upstream and downstream will be verified.

We have identified four core genes (ITGB1, CCL2, STRN3, and EDN1) potentially associated with HIF-1A in the pathogenesis of cervical cancer. Furthermore, our findings suggest that these genes may exert their influence on the progression of cervical cancer through diverse biological functions and pathways. The aforementioned findings offer novel insights into the etiology and therapeutic strategies for cervical cancer as well as other malignancies.

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Data availability statement

Data included in article/supp. material/referenced in article.

CRediT authorship contribution statement

Zhenyu Li: Writing - original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ran Wei:** Formal analysis, Data curation. **Shunyu Yao:** Formal analysis, Data curation. **Fang Meng:** Resources, Formal analysis, Data curation. **Lingsuo Kong:** Funding acquisition, Resources, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24664.

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