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Research Article

Activating Transcription Factor 5; A Potential Prognostic Molecular Biomarker for Cervical Cancer (CC)

Running Title: ATF5 Prognostic Marker for Cervical Cancer

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Abstract:

Background: Prognostic biomarkers with high sensitivity and specificity that accurately predict disease prognosis are crucial for influencing cancer patients' treatment and disease progression choices. This research attempts to determine ATF5's cervical cancer prognostic value and find a theoretical basis for cervical cancer treatment.

Method: This study began with a survival analysis, then univariate and multivariate analyses using transcriptomics, methylation, and TCGA ATF5 clinical data. Second, ATF5's role in cervical cancer development was explored using GO, KEGG, and TIMER databases. We assessed ATF5's cervical cancer treatment potential using CMap, a drug development tool.

Results: After conducting several analyses, we discovered that high levels of ATF5 mRNA expression and low levels of methylation were beneficial for patients with cervical cancer prognosis. An important finding is that ATF5 may promote apoptosis in cervical carcinoma cells. In addition, we assessed the therapeutic potential of four small molecules against cervical cancer. ATF 5 function as a tumor suppressor gene in cervical cancer has been demonstrated for the first time using numerous databases and bioinformatics techniques from multiple dimensions, which is a novel aspect of this research. Moreover, the finding that ATF5 is a biomarker that can contribute to the apoptosis of cervical cancer cells and predict a favorable prognosis for the disease was an additional innovation. In the future, ATF5 can be used as a therapeutic target and to develop anti-cancer medications.

Keywords: ATF5; ATF5Mrna; Cervical Cancer; Prognostic Biomarker; Molecular Biomarker

Introduction

Cervical cancer (CC) is the fourth most prevalent gynecological cancer in females. Each year, more than 500,000 women are diagnosed with CC, and more than 300,000 die from it [1]. Currently, more than 85 percent of CC deaths occur in low- and middle-income nations. Tragically, cervical cancer kills more women in the developing world than any other malignancy, where it accounts for 12 percent of female cancer [2, 3]. Human papillomavirus infection is almost always the primary cause of cervical cancer (Humans 2007). Human papillomavirus (HPV) types are divided into high-risk and low-risk strains based on their ability to cause cancer. Low-risk human HPV strains could be asymptomatic or induce anogenital warts, whereas high-risk human HPV strains are carcinogenic. Nearly 99 percent of precancerous lesions (cervical dysplasia) and cervical cancers are caused by high-risk HPV infections, such as HPV 16 and HPV 18 [4]. As a result of the identification of HPV vaccination and the gradual popularity of screening programs, the incidence and mortality of CC have decreased significantly over the past few decades in high-income countries. However, this is not the case in developing nations, where the majority of CC patients reside [5]. The prolonged preinvasive phase of CC renders it a preventable disease. If rigorous screening is utilized, early diagnosis and appropriate treatment are possible [6]. Currently, chemotherapy, radiation therapy, and radical hysterectomy are the cornerstones of CC treatment. The lack of clarity regarding the pathogenesis of CC and the absence of a highly specific and

sensitive target for disease diagnosis and treatment is a major contributor to the poor prognosis of cervical cancer patients. Consequently, it is crucial to identify a suitable biomarker to serve as a theoretical foundation for developing individualized patient diagnosis and treatment. Activating transcription factor 5 (ATF5) has been identified as a molecular biomarker for hepatocellular carcinoma with the ability to predict a favorable prognosis [7]. Activating transcription factor 5 resides on chromosome 19q13.3 and is a member of the basic leucine zipper (bZip) family, which also includes proteins FOS, NRF2, and CREB and plays a crucial role in oncogenesis [8]. FOS regulates cellular proliferation and apoptosis and can increase colorectal cancer risk [9]. CREB regulates cell survival, proliferation, and differentiation and acts as a protooncogene in the initiation, development, and metastasis of a variety of cancers, including prostate, breast, and non-small-cell lung cancers [10]. Several studies indicate that ATF5 is highly correlated with the pathological progression of numerous malignancies, including esophageal cancer, nasopharyngeal carcinoma, and glioma [11,12]. In addition, ATF5 participates in numerous cancer-related pathways, such as tissue differentiation and endoplasmic reticulum stress [7]. Many research studies have revealed that ATF5 plays a significant role in regulating the differentiation of various tissues, including the brain, liver, and other organs [13,14]. Additionally, ATF5 inhibits the differentiation of brain tissues, which is up-regulated in gliomas and reduces the overall survival of glioma patients. ATF5 stimulates liver tissue differentiation, which is down-regulated in hepatocellular carcinoma and contributes to a favorable prognosis for hepatocellular carcinoma patients [7]. To our knowledge, only one piece of literature asserts that differentiation is a crucially independent factor in cervical cancer [15]. By modulating the differentiation of cervix tissue, ATF5 may therefore influence the development of CC. ATF5 is regulated by CHOP and endoplasm reticulum stress, and ER stress can affect cell survival [16, 17]. Endoplasmic reticulum stress can induce cell autophagy via three signaling pathways including the IRE1-XBP1 pathway, the PERK-eIF2 pathway, and the ATF6 pathway, and autophagy can induce cell apoptosis in the early phases of cancer [18-20]. According to research, curcumin can activate endoplasmic reticulum unfolded protein response (ER UPR) sensors such as ATF6 and CHOP, which are essential for endoplasmic reticulum-mediated cell apoptosis, resulting in a decrease in the expression of the anti-apoptotic protein Bcl-2 and an increase in the expression of the pro-apoptotic protein Bax, thereby increasing the apoptosis of cervical cancer cells [21]. Therefore, we can speculate that ATF5 may influence the pathological mechanism and progression of CC by actively participating in cell differentiation and apoptosis. The role of ATF5 in the occurrence and progression of CC has not previously been reported. Thus, we conducted this research to investigate the precise mechanism of ATF5 on CC. To identify a novel and effective biomarker that can aid in the accurate diagnosis of CC, as well as a new target for cervical cancer anti-tumor therapy. This is the first study to demonstrate that ATF5 is a novel and effective prognostic marker for cervical cancer and to suggest that it may be a therapeutic target for cervical cancer. This research also provides a novel theoretical basis for cervical cancer diagnostics and anti-tumor therapy, as well as a fresh perspective on the pursuit for the development of anti-cervical cancer drugs. We identified and evaluated the expression status of ATF5 in CC using multiple databases and hundreds of samples in the current study. In addition, we investigated the regulation of ATF5 expression, the biological functions of ATF5, and a number of potential small-molecule therapeutics associated with ATF5. The results demonstrated that ATF5 mRNA functions as a tumor suppressor gene for CC and has a prospective role in predicting a favorable prognosis for patients with cervical cancer. In previous studies, it is reported that ATF5 functions as an oncogene in most malignancies. However, in this study, we testify to the carcinostatic role of ATF5 in CC for the first time, which complements the deficiency of ATF5 research in cervical cancer.

Materials and Methods

1.1 Data Collection:

The Cancer Genome Atlas (TCGA) is a publicly funded pilot project initiated by the National Institutes of Health (NIH), which attracts scientists from National Cancer Institute (NCI) and National Human Genome Research Institute (NHGRI) to create a comprehensive integrated genomics atlas for researchers to understand the biology and pathology of cancers, thereby facilitating the improvement of cancer therapy techniques and ultimately aiming to prevent neoplasia [22]. In this research, UCSC Xena (https://xenabrowser.net/) was used to collect 191 cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) samples, methylation profiles, and clinical data assessing a number of clinical factors, including age, stage, pathology, and others. Then, 191 samples' transcriptome data were retrieved from the TCGA database (https://portal.gdc.cancer.gov/). The information gathered above was combined to investigate the correlation between ATF5 expression level and clinical characteristics of CESC patients as well as the influence of methylation level and ATF5 expression level on prognosis among CESC patients. All CESC patients' clinical characteristics were outlined in (Table S1). Gene Expression Profiling Interactive Analysis (GEPIA), which is based on TCGA and GTEx data, offers immediate and customizable functions. GEPIA is a web server for cancer and normal gene expression profiling and interactive analyses. Therefore, GEPIA was used to compare the levels of ATF5 mRNA expression in samples of cervical cancer and non-tumorous tissue. In addition, we downloaded the gene profile data for GSE9750, GSE19188, and GSE67522 to verify the differences in ATF5 expression in healthy tissues and CESC issues [23, 24]. These were respectively based on the GPL 96 (Affymetrix Human Genome U133A Array), GPL 570 (Affymetrix Human Genome U133 Plus 2.0 Array), and GPL 10558-50081 (Illumina HumanHT-12 V4.0 expression bead chip) platforms. GSE9750 contained 24 normal cervical tissues and 33 cervical squamous cell carcinoma and endocervical adenocarcinoma samples, GSE19188 contained 65 normal cervical tissues and 91 CESC samples, and GSE67522 contained 17 normal cervical tissues and 25 CESC samples. Each of the aforementioned datasets was retrieved from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). It is a public repository of functional genomics data that provides users with access to investigate, download, and curate experiments and gene expression profiles. (GSE63733 2019). Human Protein Atlas (HPA) (https://www.proteinatlas.org) was subsequently utilized to further evaluate the ATF5 expression variations from protein levels in normal and malignant tissues. It is a public database that seeks to map all human proteins in cells, tissues, and organs by integrating various omics technologies. This allows scientists from both academia and industry to investigate the human proteome by acquiring the data for free.

1.1TIMER Database Analysis:

Tumor Immune Estimation Resource (TIMER) (https://cistrome.shinyapps.io/timer/) is a public database containing six modules. TIMER's incorporation of a number of sophisticated algorithms that enable users to search for correlations between immune infiltrates and genetic data from the TCGA database is advantageous to cancer immune research [25]. In this study, the relationship between the expression of ATF5 and the number of six immunological infiltrates (B cells, CD4+ T cells, CD8+ T cells, Neutrophils, Macrophages, and Dendritic cells) in the Gene

module was analyzed first. Then, using the SCNA module, the relationship between somatic CNA and immunological infiltrate frequency was evaluated. Observed the relationship between the expression of ATF5 and three immune checkpoint genes, PDCD1 (PD-1), CD274 (PD-L1), and PDCDLG2 using the Correlation module. (PD-L2).

1.2 Gene Ontology Enrichment Analysis and KEGG Pathway Analysis:

Gene ontology (GO) is the most exhaustive source of information on gene function in the world. It is commonly used for analyzing gene products and supporting biological hypotheses. Gene ontology enrichment analysis (GOEA) is a revolutionary technique for exploring gene function that has the potential to identify both upregulated and downregulated GO terms [26].

Kyoto Encyclopedia of Genes and Genomes (KEGG) was established by the Kanehisa Laboratory at Kyoto University in Japan in order to predict and calculate complex biological behaviors and cellular pathways. KEGG includes network information, genetic information, and chemical information in its comprehensive database. It is subdivided into sixteen primary databases, including the KEGG PATHWAY database [27]. KEGG pathway analysis and gene ontology enrichment analysis was used in this study to explore the function of ATF5 in biological mechanisms and potential pathways in which ATF5 may be implicated in CC. First, based on the median level of ATF5 expression, we divided patients with CESC into two groups: high ATF5 expression and low ATF5 expression. Second, we chose genes whose expression varied between the two groups. Thirdly, GO and KEGG analyses were performed to identify the biological processes and KEGG pathways associated with these differentially expressed genes.

1.3 Connectivity Map (CMap) For cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC)

A connectivity Map, also known as CMap (https://portals.broadinstitute.org/CMap/), was utilized to determine the functions of small molecules in the treatment of cervical cancer. It is one of the most significant tools in the field of drug repositioning and endeavors to identify medications with putative efficacy for a disease characterized by differential gene expression [28]. The up-and down-regulated ATF5-related genes were then uploaded to the Connectivity Map website to obtain the associated small molecule compounds. The following step was to determine the positively linked molecular therapeutics (P 0.01 and enrichment > 0) for CESC.

1.4 Statistical Analysis:

All data acquired for this study were analyzed using R software (Version 3.6.1). First, the Wilcoxon rank sum test was used to compare the expression levels of ATF5 in tumor-related and non-tumor-related conditions. Second, a chi-square test was performed to assess the relationship between ATF5 and clinical characteristics. Thirdly, Kaplan-Meier survival analysis was utilized to determine the effect of ATF5 expression or methylation level on the overall survival of patients. Fourthly, univariate and multivariate Cox analyses were used to determine the risk factors for CC. To further support the effect of ATF5 on cervical cancer, a Pearson correlation coefficient-based co-expression analysis was performed to identify genes associated with ATF5

Results

2.1ATF5 expression level in normal and tumor tissues of cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) patients

According to reports, the expression of genes that regulate the pathological process of cancer differs between va rious tumor tissues and their corresponding normal tissues. Multiple varieties of cancer (glioma, breast carcinoma, ovarian carcinoma, hepatocellular carcinoma, etc.) have been associated with ATF5 mRNA [11, 12]. Given that gynecologic malignancies are the focus of our team's research, the purpose of this study is to investigate the association between the ATF5 gene and CC.

To determine the difference in ATF5 expression between normal tissues and CSEC tissues, we utilized normal tissues and CESC tumor tissues from Gene Expression Profiling Interactive Analysis (GEPIA) and discovered that the expression level of ATF5 in various tumors. ATF5 is substantially elevated in cervical tumor samples compared to non-tumor samples. red represents higher expressiveness while green represents lower expression. (Figure1A). In addition, three sets of genes from the Gene Expression Omnibus database (GSE9750, GSE19188, and GSE67522) also yielded comparable results (figure1(B-D). Then, immunohistochemical samples of cervical carcinoma were retrieved from the Human Protein Atlas database. Immunohistochemistry (IHC) is a technique that uses microscopy to visualize cellular components like proteins or macromolecules in tissue samples. IHC's visual output shows the target-protein's presence and location in distinct cell types. After analyzing the three samples, a significant difference in ATF5 protein level was found between them, indicating that the protein level of ATF5 in normal tissues was significantly higher than that in both cervix adenocarcinoma and squamous cell carcinoma. (figure2(A-C). ATF5 may serve a regulatory role in the pathogenesis of CC based on its differential expression in the disease. Different pathological disease states will influence the associated clinical symptoms, making it crucial to research the clinical aspects of CC.

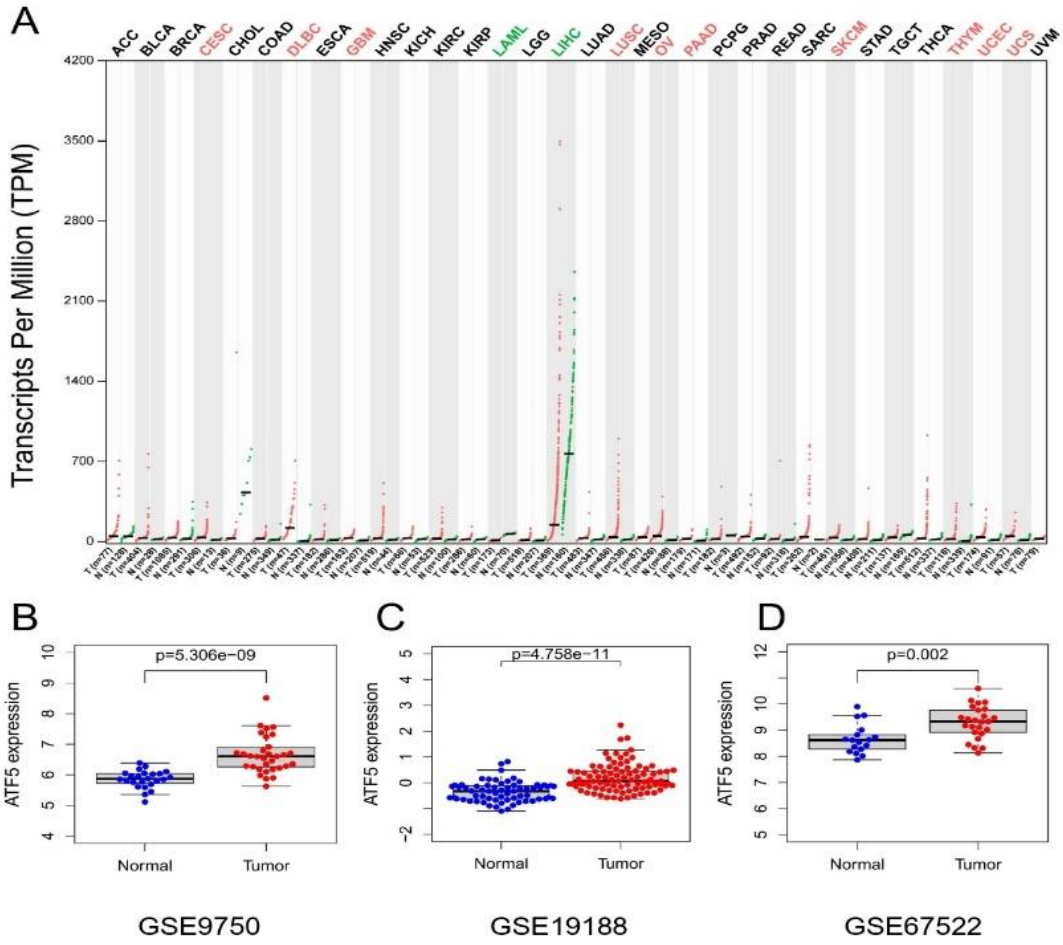

Figure 1 (A): ATF5 expression levels in CESC patients' normal and malignant tissues from TCGA. The levels of ATF5 expression in tumor tissues are significantly higher than in normal tissues. (B-D). The 3 gene sets (GSE9750, GSE19188, and GSE67522) all indicate that the level of ATF5 expression in tumor cells is higher than in normal cells.

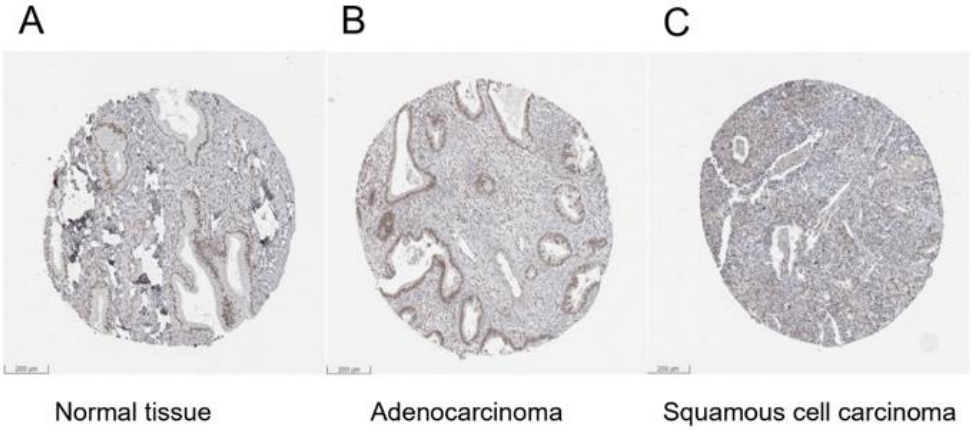

Figure 2(A-C): Normal cervix tissues **(A)** had a considerably higher ATF5 protein level than cervical adenocarcinoma **(B)** and squamous cell carcinoma **(C)**, according to immunohistochemical analysis.

1.3 The relationship between clinical characteristics and ATF5 expression

Since cancer associated genes and tumor clinical characteristics are closely correlated. In order to comprehend the precise functional mechanism of a gene in the development of a particular malignant tumor, it is necessary to analyze the gene and its clinical characteristics. In the current research, we explored the correlation between ATF5 mRNA expression and several clinical characteristics, such as age, clinical stage, neoplasm histologic grade, and pathologic tumor, node, and metastasis (TNM) stage, as shown in (Table S1). ATF5 correlates substantially with age, clinical stage, and pathologic T stage. Patients older than 46 have higher levels of ATF5 expression than those younger than 46 ($p < 0.05$; Figure 3A). In addition, the expression level of ATF5 mRNA was substantially lower in clinical stage IV than in clinical stage III ($p < 0.05$ Figure 3B). In accordance with the pathologic T stage, ATF5 expression decreased substantially with increasing T stage ($p < 0.05$ figure 3C) However, ATF5 and the remaining clinical characteristics (neoplasm histologic grade, pathologic N stage, and pathologic M stage) did not correlate significantly ($p \geq 0.05$) (Figure S1). Even if the p-value is not statistically significant, there is a trend of a negative association between ATF5 and the pathologic N and M stages. It is the first time to explore the relationship between the ATF5 gene and the clinical characteristics of cervical cancer, suggesting that ATF5 may be a tumor suppressor gene that inhibits oncogenesis and tumor metastasis. However, it is insufficient to demonstrate ATF5's role in predicting the prognosis of cervical cancer.

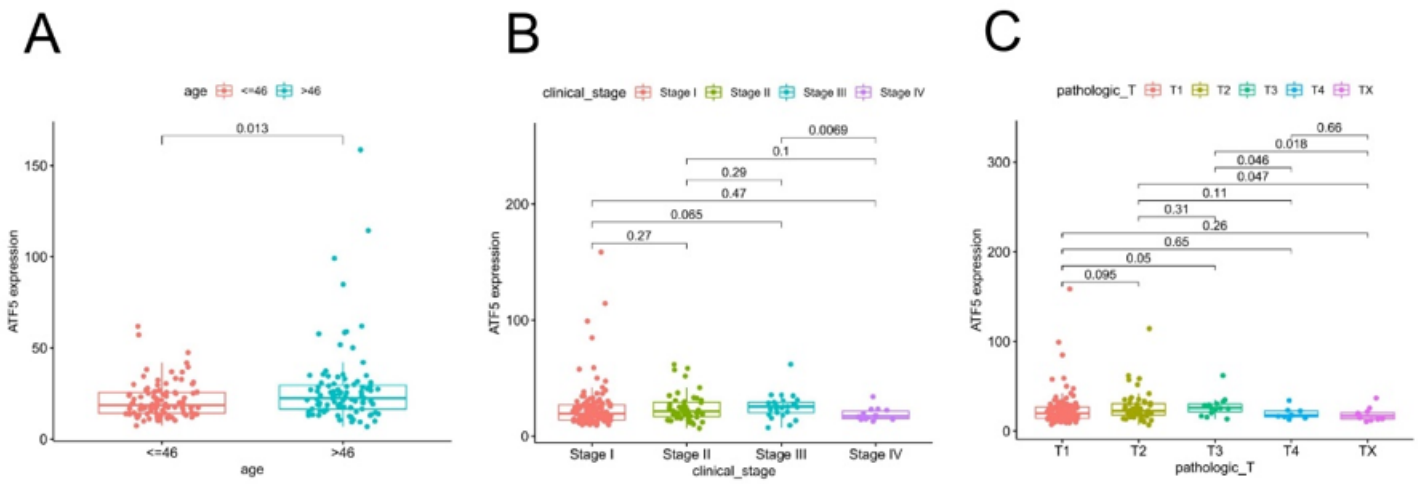

Figure 3(A-C): Several clinical characteristics, including age, clinical stage, neoplasm histologic grade, and pathologic TNM stage, are displayed alongside ATF5 expression. Age, clinical stage, and pathologic T stage have significant associations with ATF5. Patients over 46 have higher ATF5 expression levels than patients under 46 (p0.05). **(A)**. Clinical stage IV demonstrated significantly reduced ATF5 expression levels than clinical stage III (p0.05) pathological stage T1 is significantly higher while T4 is considerably lower. **(B-C)**. (The tumor, node, and metastasis (TNM)).

1.4 The prognostic role of ATF5 for cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) patients

In light of the foregoing description, a K-M survival analysis was conducted to investigate the further role of ATF5 in predicting the prognosis of CESC patients (Figure 4A). Included were 96 samples with low ATF5 expression and 95 samples with high ATF5 expression. Comparing the experimental outcomes of the two groups revealed that patients with high levels of ATF5 mRNA expression had better results compared than patients with low levels of ATF5 mRNA expression. This finding suggests that ATF5 expression level may be associated with a favorable prognosis for CESC patients, and therefore ATF5 may be used as a prognostic predictor for CESC. Subsequently, univariate and multivariate analyses were conducted to investigate the validity and applicability of ATF5's prognostic role for CESC. High ATF5 expression was found to be a low-risk factor for cervical cancer by univariate analysis, whereas higher clinical stage, pathologic N stage, and pathologic T stage were high-risk variables (Figure 4B). Multivariate analysis also revealed an independent relationship between ATF5 mRNA and survival time (Figure 4C). In conclusion, the aforementioned results suggest that ATF5 is an independent risk factor for CC. High ATF5 expression can enhance a patient's prognosis, making it possible to predict the clinical prognosis of cervical cancer patients. However, evaluating ATF5's prognostic value for CC from a single perspective is insufficient; it is necessary to discuss ATF5's prognostic value from a variety of perspectives.

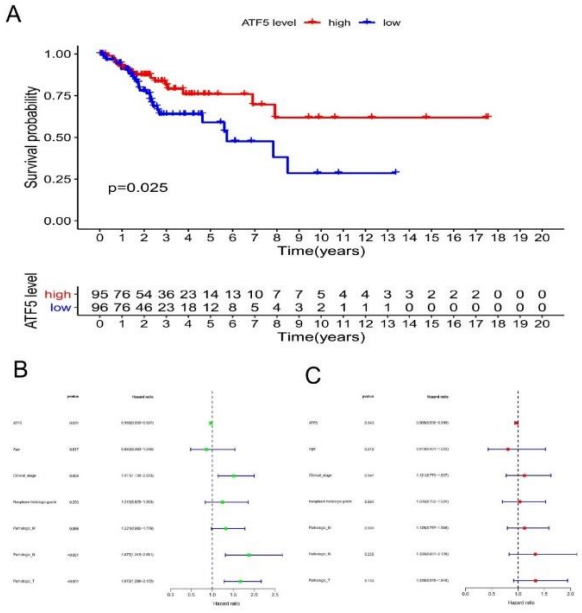

Figure 4(A-C): Illustration of the role of ATF5 in predicting the prognosis of CESC patients based on TCGA data survival analysis. (C) According to univariate analysis, a higher ATF5 expression level was a low-risk factor for cervical cancer, (B) whereas high level of clinical stage, pathologic N stage, and pathologic T stage were risk factors. (A) ATF5 mRNA was found to be independently related to survival time in a multivariate analysis.

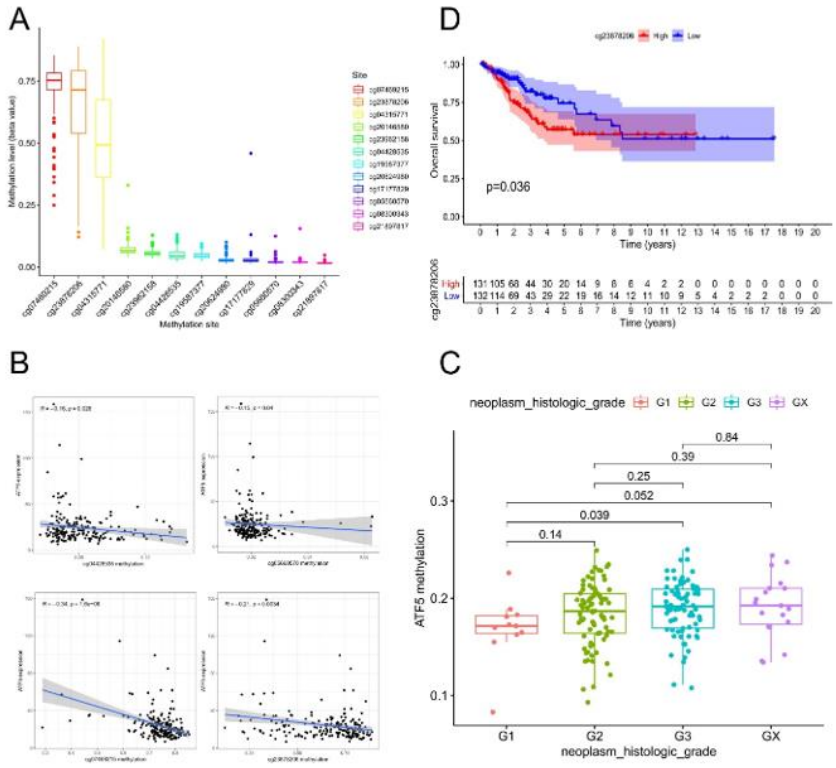

Figure 5(A-D): (A). demonstrates the importance of ATF5 methylation for prognosis in CESC patients.
(B). Inverse association between (cg04428535, cg05660570, cg07469215, and cg23878206) and ATF5 expression was found using Pearson correlation analysis.
(C). indicates ATF5 methylation as well as several clinical traits. The tumor grade III had significantly more ATF5 methylation than the tumor grade I did, according to histopathological classification.
(D). Patients with high levels of methylation have substantially worse survival rates than those with lower levels of methylation, according to K-M plots.

1.6 Gene Ontology Enrichment Analysis and KEGG Pathway Analysis

In the previous two paragraphs, we have demonstrated how ATF5 influences the prognosis of cervical cancer. The next step is to investigate how the specific mechanism of ATF5 influences the prognosis of cervical cancer. Consequently, GO enrichment analysis and KEGG pathways enrichment were used to obtain a deeper understanding of the molecular mechanisms by which ATF5 affects CC as illustrated in (Figure6A). Neutrophil activation, neutrophil-mediated immune regulation, interferon-gamma response, and interferon-gamma-mediated signaling pathway biological processes involve ATF5 mRNA. In addition, **Figure 6B** demonstrates that ATF5 is involved in the apoptotic pathway, which has been associated with the progression of cancer [30-32]. These results suggest that ATF5 and immunity have a substantial relationship. Immunotherapy is a novel and rapidly developing anti-tumor therapy that has been a hotspot for research in the field of tumor therapy. To make this study more scientific, credible, and comprehensive, we must investigate further the correlation between ATF5 and immune cells.

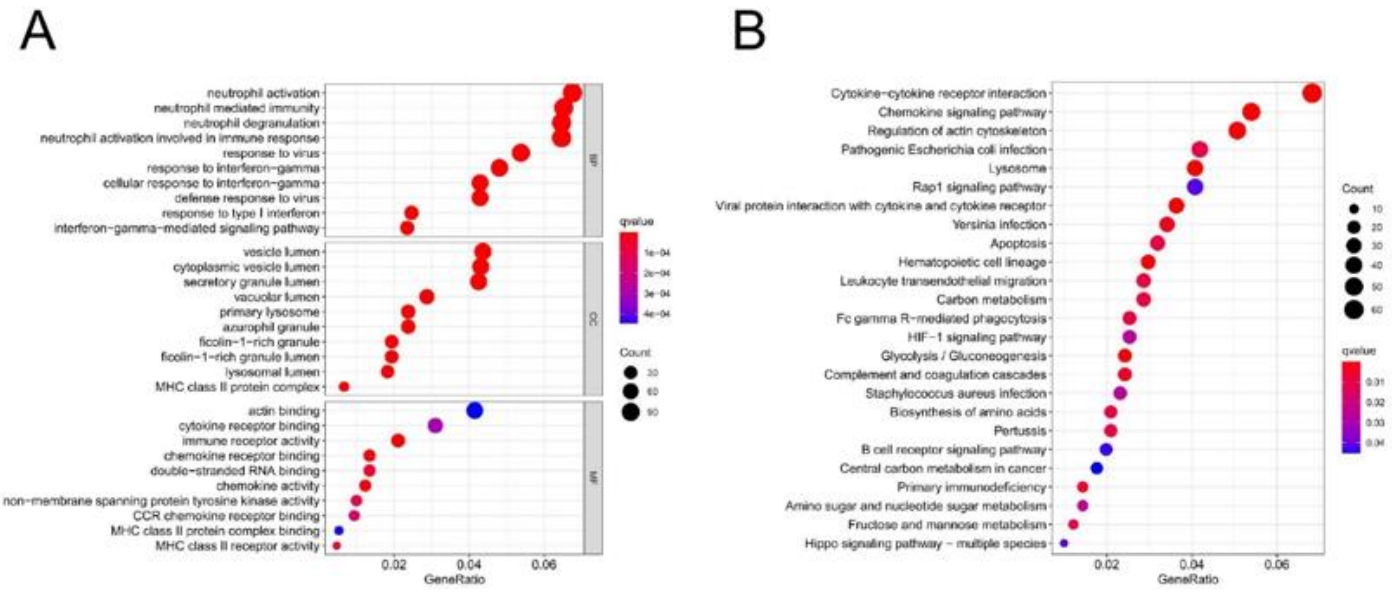

Figure 6(A-B): The biological functions of the ATF5 mRNA in immune-related biological processes, such as neutrophil activation, neutrophil-mediated immune control, interferon-gamma response, and interferon-gamma-mediated signaling pathway biological processes, are illustrated in **(A)** ATF5's involvement in the apoptotic pathway, which has been connected to the development of tumors, is demonstrated in **(B)**.

1.7 The Relationship Between ATF5 and Immune Infiltration

As stated in the preceding paragraph, ATF5 is implicated in immune-related biological processes. The relationship between ATF5 and immunological infiltrates was investigated using TIMER analysis. (Figure 7A) demonstrates that ATF5 expression is positively associated with the immunological infiltration of B cells, CD8+ T cells, CD4+ T cells, neutrophil cells, and dendritic cells. Additionally, arm-level deletion and high amplication are linked to CESC immune cell infiltration. ATF5 expression levels positively relate with the expression of PDCD1, CD274, and PDCD1LG2 shown in (Figure 7B, Figure 7C).

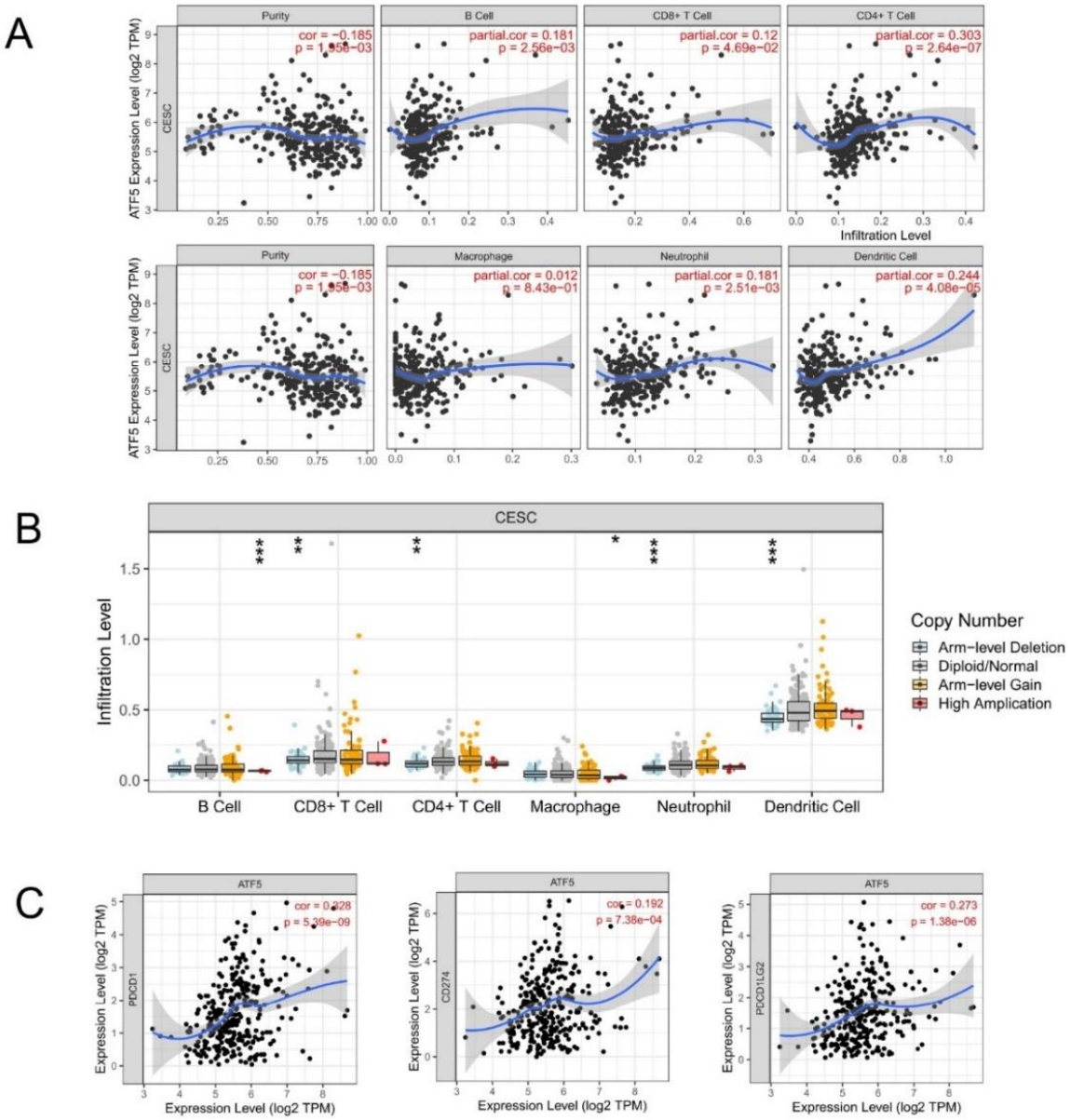

Figure 7(A-C): (A) demonstrates that the expression of ATF5 is positively correlated with the invasion of B cells, CD8+ T cells, CD4+ T cells, neutrophils, and dendritic cells.
(B). Arm-level deletion and high amplication are linked with CESC immune cell infiltration.
(C). There is a positive correlation between the expression levels of ATF5 and the expression levels of PDCD1 p value= 5.39e-09, CD274 p value=7.38e-04, and PDCD1LG2 p value=1.38e-06.

1.8 Co-expression Analysis of ATF5

In the organism, a single functional gene performs numerous functions. In order to determine how ATF5 influences the transcript of other genes and to represent the rigorous and scientific nature of the research, it was decided to carry out a co-expression analysis to find genes that are strongly associated with ATF5 and, as a result, assess ATF5's indirect contribution to CC. Based on the p-value, we identified genes that were positively and negatively associated with ATF5 using co-expression analysis. Next, a circle blot of the five most negatively and positively correlated genes with ATF5 was conducted (Figure 8). (Table 1). ATF5 has positive correlations with PARM1, TRAF2, LTB, KRT36, and NINJ1, and negative correlations with ATP6V1D, PLIN3, NDFIP2, SSH3, and AHNAK, according to the results of the circle blot. Some of these genes have been identified as prognostic biomarkers for certain malignant tumors, suggesting that ATF5 may be a prognostic biomarker for CC, thereby bolstering the credibility of our study

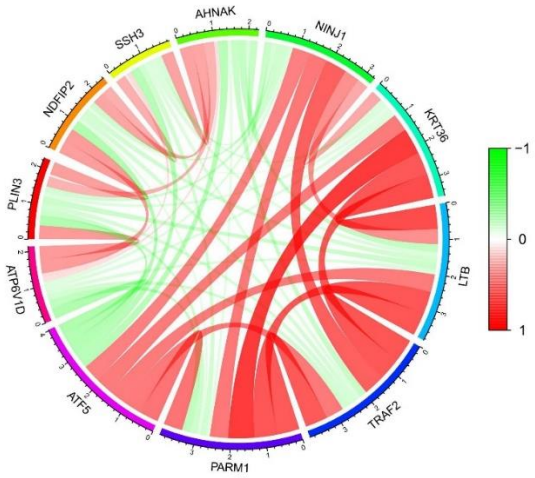

Figure 8: Illustrates the positive relationships between ATF5 and PARM1, TRAF2, LTB, KRT36, and NINJ1, but the negative correlations between ATF5 and ATP6V1D, PLIN3, NDFIP2, SSH3, and AHNAK. red represents positive relationship and green represents negative relationship.

genel	gene2	cor	p-value
ATF5	ATF5	1	0
ATF5	PARM1	0. 549	1.82E-21
ATF5	TRAF2	0. 521	3. 64E-19
ATF5	LTB	0.516	1.01E-18
ATF5	KRT36	0.512	1.86E-18
ATF5	NINJ1	0.499	1.92E-17
ATF5	ATP6V1D	-0. 301	9.34E-07
ATF5	PLIN3	-0. 295	1.68E-06
ATF5	NDFIP2	-0.292	2.02E-06
ATF5	SSH3	-0. 273	9.83E-06
ATF5	AHNAK	-0. 271	1.17E-05

Table 1: The gene co-expression with ATF5.

1.9 Identification of Potential Drugs for cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) Treatment

The ultimate goal of cancer research is to discover effective and specific chemotherapy drugs or other effective treatments to eradicate cancer, reduce the incidence and mortality rates of the disease, and improve patient prognosis. Consequently, using co-expression analysis, we screened for up- and down-regulated ATF5-related genes and uploaded them to the CMap website in order to identify the associated small molecule compounds. Using the shear standard, four small molecular compounds were identified alsterpaullone, camptothecin, doxorubicin, mitoxantrone these could be used as CESC therapy drugs as shown in (Figure 9 A-D). The 2D and 3D structures of these four compounds as well as pertinent information were obtained from the PubChem website. After additional investigation, it is anticipated that these screening drugs will be used to treat cervical cancer in the future

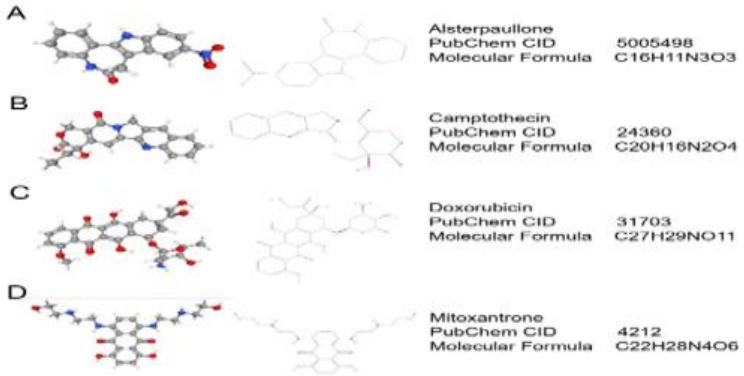

Figure 9 (A-D): Four potential drug molecules that could be used to treat cervical cancer patients are shown in their 2D and 3D structures and chemical formulas.

2. Discussion

CC is a well-known malignancy with a high incidence of morbidity and mortality. Despite the availability of numerous treatments, such as surgery, chemotherapy, and radiotherapy, a significant number of patients experience poor outcomes due to recurrence and metastatic characteristics [33]. Consequently, it is crucial to identify a biomarker that can predict the prognosis of CC early on. ATF5 has been identified as a biomarker for several types of cancer [7, 11, 12]. However, prior research had not studied the role of ATF5 in CC. This is the first study to characterize the prognostic and therapeutic significance of ATF5 in CC.

In order to conduct a complete and exhaustive analysis of the association between ATF5 expression and CC, the current study utilized several databases containing hundreds of samples from multiple dimensions. Oncogenes and tumor suppressor genes are expressed differentially in normal versus tumor tissues of specific cancers [34, 35]. Consequently, we examined the expression of ATF5 in cervical cancer using three databases. In the GEPIA database, we discovered that the quantity of ATF5 mRNA expression varies between tumor types. For example, ATF5 mRNA expression is down-regulated in tumor tissues of Acute Myeloid Leukemia (LAML) and Liver hepatocellular carcinoma (LIHC) compared to adjacent non-tumor tissues, whereas ATF5 mRNA expression is up-regulated in tumor tissues of cervical squamous cell carcinoma and endocervical adenocarcinoma, Lymphoid Neoplasm Diffuse Large B-cell Lymphoma (DLBC),Glioblastoma multiforme (GBM), Lung squamous cell carcinoma (LUSC), Ovarian serous cystadenocarcinoma (OV), Pancreatic adenocarcinoma (PAAD), Skin Cutaneous Melanoma (SKCM), Thymoma (THYM), Uterine Corpus Endometrial Carcinoma (UCEC),and Uterine Carcinosarcoma (UCS). Then, to evaluate the expression level of ATF5 in cervical cancer, we utilized the GEO database and the HPA database to compare the gene and protein levels of ATF5 expression between cervical cancer tissues and normal tissues. Surprisingly, the GEO database led us to the same conclusion as the GEPIA database: the expression level of ATF5 mRNA is higher in tumor tissues than in tumor-free tissues. However, we discovered an anomaly in the HPA database: the protein quantity of ATF5 expression in tumor tissues is lower than in normal tissues. First, CESC tumor tissues in the GEPIA and GEO datasets include adjacent normal tissues and other components, suggesting they are not pure tumor tissues. ATF5 mRNA levels may be elevated. Epigenetics regulates gene expression, and methylation is a key component of epigenetics [36,37,38,39]. This study demonstrated a negative correlation between the methylation level of ATF5 and the level of mRNA expression. However, only the methylation data of patients with cervical cancer are available in the TCGA database, while the methylation data of normal cervical samples are unavailable. Methylation levels and the expression of ATF5 mRNA may have distinct associations in normal cervical samples. The purpose of this study is to investigate the relationship between ATF5 and CC prognosis in an effort to identify a biomarker with significant value for the early detection and treatment ofCC. Therefore, it is crucial to examine the relationship between ATF5 and the malignant characteristics and overall survival time of CC (Fig3a, Fig3b, Fig3c). Then, to further elucidate the prognostic value of ATF5 in CC, we evaluated 191 samples from TCGA database patients withCC. The results suggested that increased ATF5 expression may enhance the prognosis of patients withCC. High ATF5 expression, similar to ATF5 expression in HCC, is indicative of a favorable prognosis for HCC patients [32, 40]. In normal cellular stress response systems help cells survive adverse events and enable them to regain homeostasis. In order to avoid apoptosis and lessen a condition of ongoing cellular stress, cancer cells frequently co-opt these same mechanisms. Under various stress circumstances, activating transcription factor 5 is upregulated and is overexpressed in a variety of cancer [41]. To avoid bias and guarantee the precision of the study, univariate and multivariate analyses were conducted to determine whether ATF5 is reliable and accurate in predicting the excellent prognosis of cervical cancer. The results suggested that ATF5 could be used as an independent biomarker to predict a favorable prognosis in patients withCC. Methylation is one of the most significant factors that affect gene expression. DNA methylation is generally more stable than gene expression in determining the prognosis of cancer patients [42-44]. To ensure the scientific validity and reliability of the investigation, 12 methylation sites were retrieved from the TCGA database. Following a series of analyses, we discovered that ATF5 expression was negatively correlated with methylation level and that hypomethylation was associated with an improved prognosis for patients with cervical cancer, confirming the earlier conclusion. We validated ATF5 as a prognostic biomarker for cervical cancer from an epigenetics perspective, which is also one of the study's novelties. Based on the information presented above, it is reasonable to presume that ATF5 will be a future treatment target for cervical cancer. To effectively target ATF5 for the treatment of cervical cancer, a thorough evaluation of ATF5's role in the pathogenesis of cervical cancer is necessary. As a result, we utilized GO and KEGG pathway analysis to determine how ATF5 inhibits cervical cancer. Significant discovery: ATF5 is implicated in the apoptotic process of CC. Unrestrained proliferation is one of the most essential characteristics of cancer cells. Apoptosis is a form of predetermined cell demise. Promoting cancer cell apoptosis can reverse cancer cell proliferation and invasion, thereby accomplishing the objective of inhibiting and treating cancer [45]. For instance, targeting apoptotic inhibitors has progressively become a new cancer treatment in recent years [46-49]. Continuous activation of caspases induces apoptosis in mammals, whereas ER stress activates caspases to induce apoptosis. ATF5 is also implicated in the association between ER stress and cell survival [7]. Therefore, there are numerous indications that ATF5 can induce apoptosis in CCcells. In the future, it may be possible to treat CC by inducing apoptosis in cervical cancer cells by targeting ATF5. GO analysis indicates that ATF5 is involved in immune-related biological processes. In recent years, immunotherapy has emerged as a novel and effective cancer treatment method [50]. Consequently, we utilized the TIMER database to investigate further the relationship between ATF5 and immunity. Numerous immune cells were discovered to have a significant positive correlation with ATF5. It is important to emphasize that T lymphocytes have a tumor-inhibiting influence, as previously reported in numerous publications [51]. In addition, it was recently discovered that B-cell infiltration is a critical differentiating factor for enhancing survival rate, which is a remarkable accomplishment [52]. Immunologically, these findings provide theoretical support for the antitumor activity of ATF5.

In addition, we examined the effect of ATF5 copy number variation on immune cell infiltration from a genetic standpoint, thereby enhancing the depth and scope of the study. Co-expressed genes frequently participate in the same pathway or have similar behaviors and functions. Co-expression analysis employs a large quantity of gene expression data to establish the correlation between gene components in order to assess gene function [53]. In order to indirectly confirm ATF5's anti-tumor function at the gene level, we continued to undertake gene co-expression analysis. We searched for the five genes most positively associated with ATF5 and the five genes most negatively associated with ATF5. Many of them are associated with the development and progression of cancer and serve as biomarkers in numerous

tumors. PARM1 inhibits tumor growth in colorectal cancer (CRC). Patients with colorectal cancer who have minimal PARM1 expression have a poorer prognosis. PARM1 regulates the BMP/Smad signaling pathway; when activated, CRC cells undergo apoptosis and are inhibited from proliferating [54]. NINJ1 can also inhibit the IL-6 signaling pathway, and inhibiting NINJ1 can reduce the inhibition of the IL-6 signaling pathway, thereby increasing the invasiveness of lung cancer [55]. Lastly, our findings validate ATF5's pro-apoptotic and anti-metastatic as well as anti-tumor properties. In addition, these findings raise the question of whether these co-expressed genes regulate CC. This issue presents opportunities for the targeted treatment of CC, as well as the possibility of investigating novel areas. The ultimate objective of disease research is the development of effective treatments. As drug therapy is a fundamental noninvasive treatment, the discovery of a cancer-specific drug is extremely significant. Based on the co-expression analysis, we screened multiple small molecular compounds with potential significance for the treatment of CC using the CMap website. Alsterpaullone, camptothecin, doxorubicin, and mitoxantrone are four small molecules that could be employed in CESC therapy as shown above in figure 9. Alsterpaullone has been identified as an inhibitor for the treatment of group 3 medulloblastoma, according to one study According to one study alsterpaullone can inhibits the proliferation of tumor cells in a dose- and time-dependent manner and exhibits significant cytotoxicity against HeLa cells. It can induce rapid apoptosis and block the cell cycle by activating caspase and regulating numerous apoptotic protein [56] whereas Doxorubicin has been identified as a targeted therapy for breast cancer [57, 58]. Camptothecin, a quinolone alkaloid, exhibited potent antitumor activity in vitro against Hela cells (a cervical cancer cell line), L1210 cells (mouse lymphocytic leukemia cells), and rodents, as well as efficacy against a variety of malignancies, including gastric cancer, rectal cancer, and leukemia [59]. Camptothecin inhibits both cleavage and religation reactions of DNA replication, thereby halting cancer cell proliferation [60]. Whereas mitoxantrone induces single- and double-stranded disruptions and inhibits DNA repair by intercalating with the DNA molecule. Mitoxantrone suppresses B and T lymphocytes and macrophages [61]. Even though these drugs have not been linked to CC, based on the functional principle of CMAP, it is likely that they will be used to treat CC specifically. To develop drugs for the treatment of CC, however, pharmaceutical and associated professional researchers must conduct additional studies. Even though this study utilized a large quantity of data and revealed for the first time from multiple perspectives that ATF5 is a tumor suppressor gene of cervical cancer and a potential treatment target for cervical cancer, there are still open questions. Due to the absence of normal tissue methylation data in the TCGA database, we did not compare methylation levels between cervical cancer tissues and normal tissues in this study. However, we did conduct a relatively comprehensive analysis of the correlation between ATF5 methylation levels and cervical cancer prognosis in tumor tissues. Second, at the conclusion of the study, we eliminated several potential drugs for the treatment of cervical cancer. However, because we are not pharmaceutical experts, another drawback of our study is that we did not do additional research on drug function and mechanism. Conversely, offer fresh perspectives and impetus to researchers working on drug development.

Conclusions

This study is innovative because it is the first to integrate a large quantity of data to demonstrate that ATF5 is a tumor suppressor of cervical cancer in multiple dimensions and that its high expression can improve the prognosis of patients with cervical cancer.

The discovery of the ATF5 mechanism implicated in the regulation of cervical cancer was an additional breakthrough. This discovery provides a useful target for the treatment of cervical cancer and offers new hope for halting the progression of the disease, curing it, and improving the quality of life for cervical cancer patients.

Declaration

Ethical approval and consent

The data utilized in this study was acquired exclusively from publicly accessible databases, and no human subjects were involved in the research process. Thus, ethical approval was not required.

Consent for publication

Not applicable

Conflict of Interest

“No author involved in this study declares any conflict of interest."

Author contributions

Conceptualization: QA and GJY conceived and developed the research idea, formulated the hypothesis, and designed the study.

Methodology: QA, GJY, XHQ contributed to the methodology by planning and executing the experiments, collecting data, and analyzing the results.

Investigation: GJY, AQZ, SHZ performed observations, and collected data.

Writing: Original Draft Preparation: QA, AQZ wrote the initial draft of the manuscript, with contributions from AQZ, SHZ in reviewing and editing the text and preparing figures and tables

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Data and material Availability

Data and material used in this study is provided in supplementary files.

Abbreviations:

1 ATF: Activating transcription factor.
2. CSEC: Cervical squamous cell carcinoma and endocervical adenocarcinoma
3. HPV: Human papilloma virus
4. GEPIA: Gene Expression Profiling Interactive Analysis
5. TCGA: The Cancer Genome Atlas
6. TIMER: database Tumor Immune Estimation Resource database
7. GO: Gene Ontology
8. C Map: Connectivity Map
9. GOEA: Gene ontology enrichment analysis
10. HPA: Human Protein Atlas
11. GEPIA: Gene Expression Profiling Interactive Analysis
12. GEO: Gene Expression Omnibus database
13. ER: stress endoplasmic reticulum stress
14. CC: Cervical cancer
15. TNM system the tumor, node, and metastasis system
16. LAML: Acute Myeloid Leukemia
17. LIHC: Liver hepatocellular
18. DLBC: Lymphoid Neoplasm Diffuse Large B-cell Lymphoma
19. GBM: Glioblastoma multiforme
20. LUSC: Lung squamous cell carcinoma
21. OV: Ovarian serous cystadenocarcinoma

22. PAAD: Pancreatic adenocarcinoma
23. SKCM: Skin Cutaneous Melanoma
24. THYM: Thymoma
25 UCEC: Uterine Corpus Endometrial Carcinoma
26.UCS: Uterine Carcinosarcoma

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