



## FBJ MURINE OSTEOSARCOMA VIRAL ONCOGENE HOMOLOG EXPRESSION IN COLON ADENOCARCINOMA: INSIGHTS INTO PATHOGENESIS AND CLINICAL IMPLICATIONS

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### Abstract

The study focuses on understanding the role of FBJ murine osteosarcoma viral oncogene homolog (FOS) expression and its regulatory mechanisms in colon adenocarcinoma (COAD). Analysis of FOS expression using the UALCAN dataset revealed a consistent down-regulation in cancerous cells compared to normal controls. Further exploration of FOS expression across various clinical parameters demonstrated significant down-regulation across different cancer stages, racial groups, genders, and age groups among COAD patients, suggesting its crucial role in tumor development. Validation of FOS expression using the GEPIA2.0 dataset confirmed its low expression in COAD tumors compared to normal samples. Additionally, validation analysis of FOS expression across different cancer stages showed dysregulation in all stages, with the highest expression in stage I and the lowest in stage IV. The study also investigated the promoter methylation level of FOS, revealing distinct methylation patterns across cancer stages, race groups, genders, and age groups, highlighting its association with COAD pathogenesis. Survival analysis using the KM plotter tool indicated a

significant correlation between FOS expression levels and overall and disease-free survival (OS and RFS) in COAD patients, with lower FOS expression not associated with shorter survival times. Mutational analysis using the cBioPortal platform did not reveal any common alterations in COAD samples. These findings underscore the intricate role of FOS in COAD development, emphasizing its potential as a prognostic biomarker and therapeutic target in COAD management.

**Key words:** Colon adenocarcinoma, Diagnosis, Treatment

## Introduction

Cancer is a leading cause of death worldwide, imposing significant healthcare and socio-economic burdens [1-4]. Treatment procedures for malignant growths primarily include surgery, chemotherapy, radiotherapy, targeted therapy, and immunotherapy [5, 6]. Despite these treatments, issues such as drug resistance and adverse side effects persist, resulting in unsatisfactory prognosis and survival rates [7]. Colorectal cancer/colon adenocarcinoma (COAD/CRC) is characterized by uncontrolled cell growth in the colon, rectum, or appendix. In 2020, there were an estimated 104,610 new cases of colon cancer and 53,200 deaths related to colorectal cancer in the United States [8]. Although some patients initially respond to treatments like chemotherapy and targeted therapy, those with advanced colon cancer often succumb to the disease. With over 2.2 million new cases and 1.1 million deaths, the global incidence of CRC is projected to increase by 60% by 2030 [9]. Common early detection strategies for CRC include grimo-based examination, enteroscopy, and blood-based assessments [10-12]. However, some instrument-dependent detection methods are time-consuming, labor-intensive, and costly. The main treatment options for CRC are surgery, adjuvant chemotherapy (for colon cancer), neoadjuvant radiotherapy (for rectal cancer), and molecular drugs [13]. These treatments have significant drawbacks. Previous studies indicate that less than 15% of metastatic CRC cases are suitable for surgery, with the recurrence rate of CRC exceeding 80% within three years and 95% within five years post-surgery [14]. Despite advances in CRC treatment, the 5-year survival rate for patients with this disease has not significantly improved [13]. Therefore, understanding the molecular mechanisms underlying colon cancer is crucial for developing novel treatment strategies.

FOS (Fos proto-oncogene, activator protein 1 (AP-1) transcription factor subunit) is a protein-coding gene first recognized as the cell homologue of two viral v-FOS oncogenes, which induce osteosarcoma in rodents and mice. It is also known as FBJ Murine Osteosarcoma Viral Oncogene Homolog, or cellular variants of FOS (c-FOS) [15]. The FOS gene belongs to the Fos gene family, which includes FOSB, FOSL1, and FOSL2. The FOS family is part of the AP-1 transcription family [16]. These proteins contain a conserved basic Leucine Zipper (bZIP) domain, essential for their dimerization [17, 18]. Fos proteins are expressed in various cell types and tissues, and their DNA binding requires heterodimerization. Fos protein levels are tightly regulated by both transcript and protein degradation. There are two translation-dependent mechanisms contributing to rapid mRNA degradation [19]. FOS gene translocations recognized in epithelioid hemangioma (EH) and osteoblastoma (OB) result in premature stop codons, leading to the loss of the C-terminal end of the protein. This renders the protein resistant to degradation, resulting in high expression levels within tumor cells [19, 20]. However, further exploration is needed to determine the specific impact of FOS gene rearrangements on the mechanisms of mRNA degradation [19].

## Materials and methods

### Expression analysis of FOS in COAD

UALCAN is an interactive web-based tool for in-depth analysis of cancer data, encompassing clinical information on about 31 cancer types [21]. It allows users to identify biomarkers, perform in silico validation of potential genes of interest, assess the epigenetic regulation of gene expression by promoter methylation, and conduct cancer gene expression analysis. In the current study, we analyze FOS expression in normal and COAD samples by extracting data from the TCGA platform. We also

examine FOS expression considering different clinical parameters such as patient age, gender, and race using the UALCAN database.

### Prognostic analysis

Kaplan-Meier (K-M) survival analysis of FOS for overall survival (OS) and disease-free survival (DFS) was conducted using the Gene Expression Profiling Interactive Analysis version 2 (GEPIA2) dataset [22]. The differences between FOS expression and prognosis (OS and DFS) in COAD cancer patients were obtained from the GEPIA2.0 database. In this study, GEPIA2 was employed to analyze the association between FOS expression and prognosis (OS and DFS) in COAD cancer.

### Promoter methylation analysis of FOS

We used UALCAN to analyze the promoter methylation level of FOS in COAD. The UALCAN database provides information related to RNA expression, DNA methylation, viral infection, and clinical characteristics of cancer patients [21]. Additionally, we analyzed promoter methylation data of FOS across various clinical parameters, including patient age, gender, cancer stage, and race.

### Survival analysis of FOS

The Kaplan-Meier (KM) plotter is a vital and user-friendly tool for survival analysis [23]. This online platform encompasses broad clinical information to assess the impact of specific genes on patient survival across various cancer types. KM Plotter provides intuitive Kaplan-Meier survival curves, offering insights into how gene expression correlates with patient outcomes. In this study, the KM plotter tool was used to analyze the impact of FOS dysregulation on the overall survival (OS) and disease-free survival (DFS) of cancer patients.

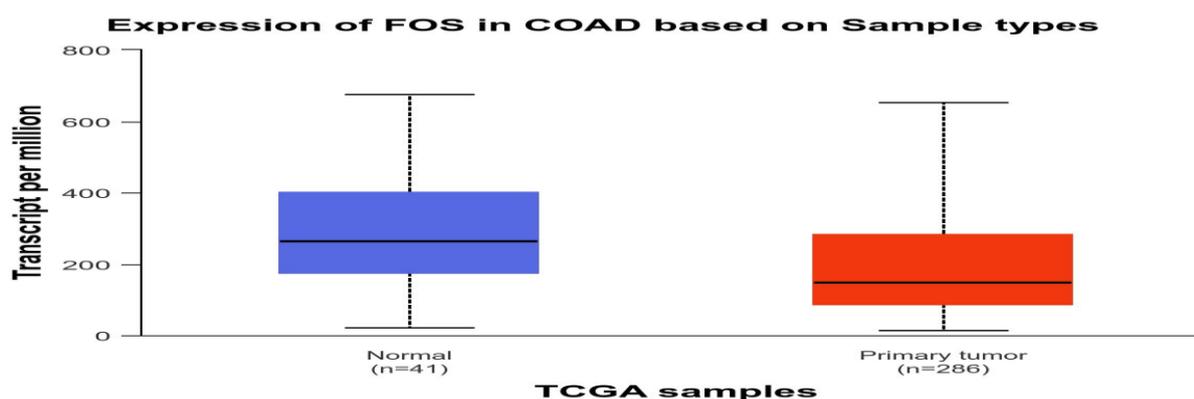
### Mutational analysis of FOS

FOS mutation features across COAD cancer were analyzed using the cBioPortal platform, which contains multidimensional cancer genomics information [24, 25]. The cBioPortal for Cancer Genomics fundamentally lowers the barriers between complex genomic data and cancer researchers, who require quick, intuitive, and high-quality access to molecular profiles and clinical characteristics from large-scale cancer genomics projects. This platform enables researchers to interpret these rich datasets into biological insights and clinical applications.

## Results

### Expression analysis of FOS in normal control and COAD samples

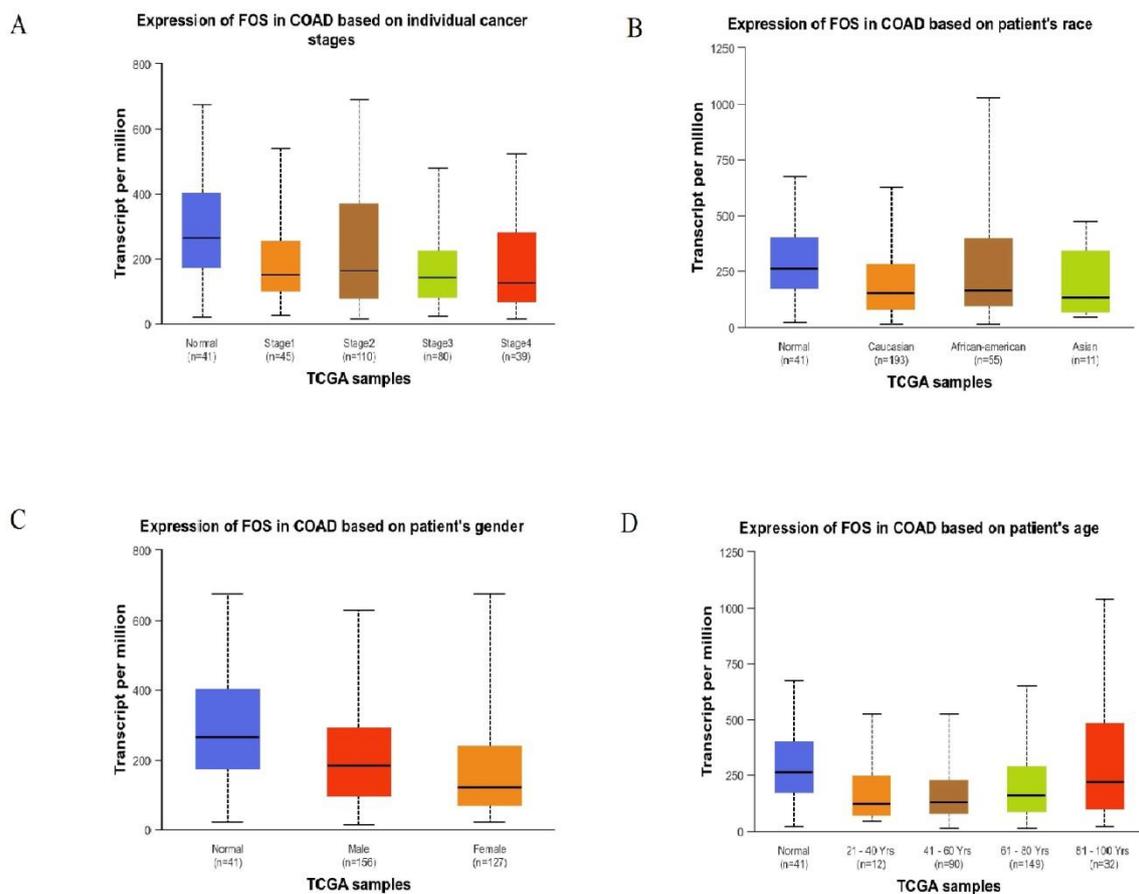
Upon utilizing the UALCAN database, our initial investigation focused on FOS expression across both normal and cancerous tissues (Figure 1). Our findings revealed significant down-regulation of FOS in COAD cancer compared to normal control samples. This notable down-regulation suggests a potential relationship between FOS expression and the proliferation of COAD cancer cells.



**Figure 1:** This figure shows the expression profiling of FOS in COAD and normal tissue samples.

### Expression analysis of FOS in COAD cancer divided based on different clinical boundaries

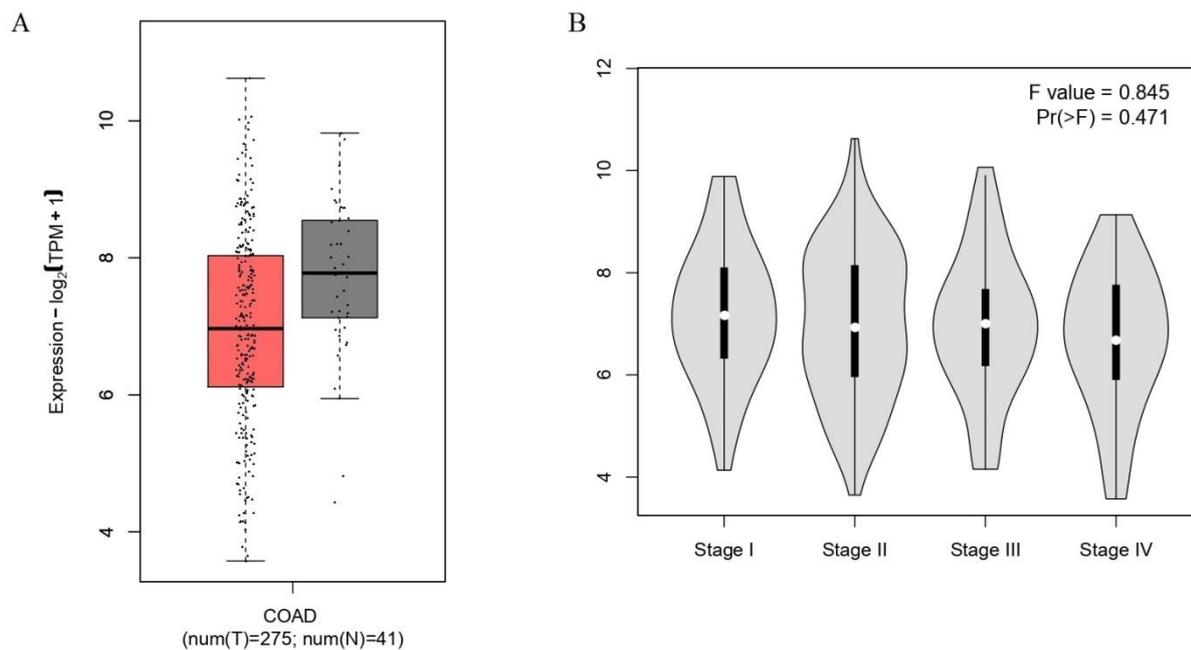
Simultaneously, we conducted an analysis of FOS expression in COAD samples across various clinical parameters, including individual cancer stages, patient race, gender, and age (Figure 2). Initially, we examined FOS expression across different cancer stages and observed a significant down-regulation of FOS in COAD across all stages compared to normal control samples (Figure 2A). Similarly, we analyzed FOS expression in COAD patients across different races, revealing consistent down-regulation of FOS in Caucasian, Asian, and African-American groups compared to normal control samples (Figure 2B). Moreover, we examined FOS expression in COAD patients by gender, showing notable down-regulation of FOS in both male and female patients compared to normal control samples (Figure 2C). Finally, we investigated the relationship between FOS expression and patient age in COAD. Our findings demonstrated down-regulation of FOS expression across various age groups among COAD patients (Figure 2D).



**Figure 2:** Expression of FOS across different clinical boundaries

### Validation of FOS on additional COAD data set

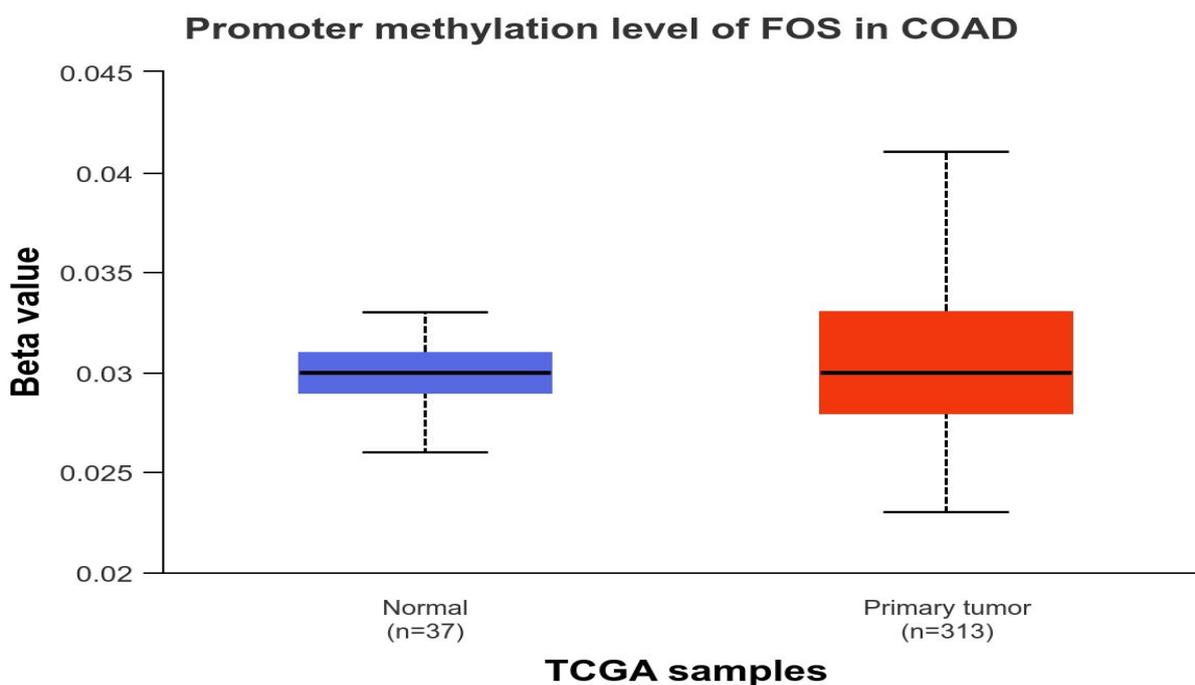
We utilized GEPIA2 to further investigate the FOS expression between COAD tumors and the corresponding normal tissues. The outcome portrayed that FOS was expressed at lower levels in colon adenocarcinoma (COAD) compared to normal control samples (Figure 3A). Furthermore, we analyzed the connection between FOS expression and pathological stages using the GEPIA2 database. The results showed that FOS expression was strongly associated with the stages of patients with colon adenocarcinoma (COAD). Moreover, in COAD, FOS had the highest expression.



**Figure 3:** Validation of FOS across different stages of COAD

### Promoter methylation of FOS in COAD and normal control samples

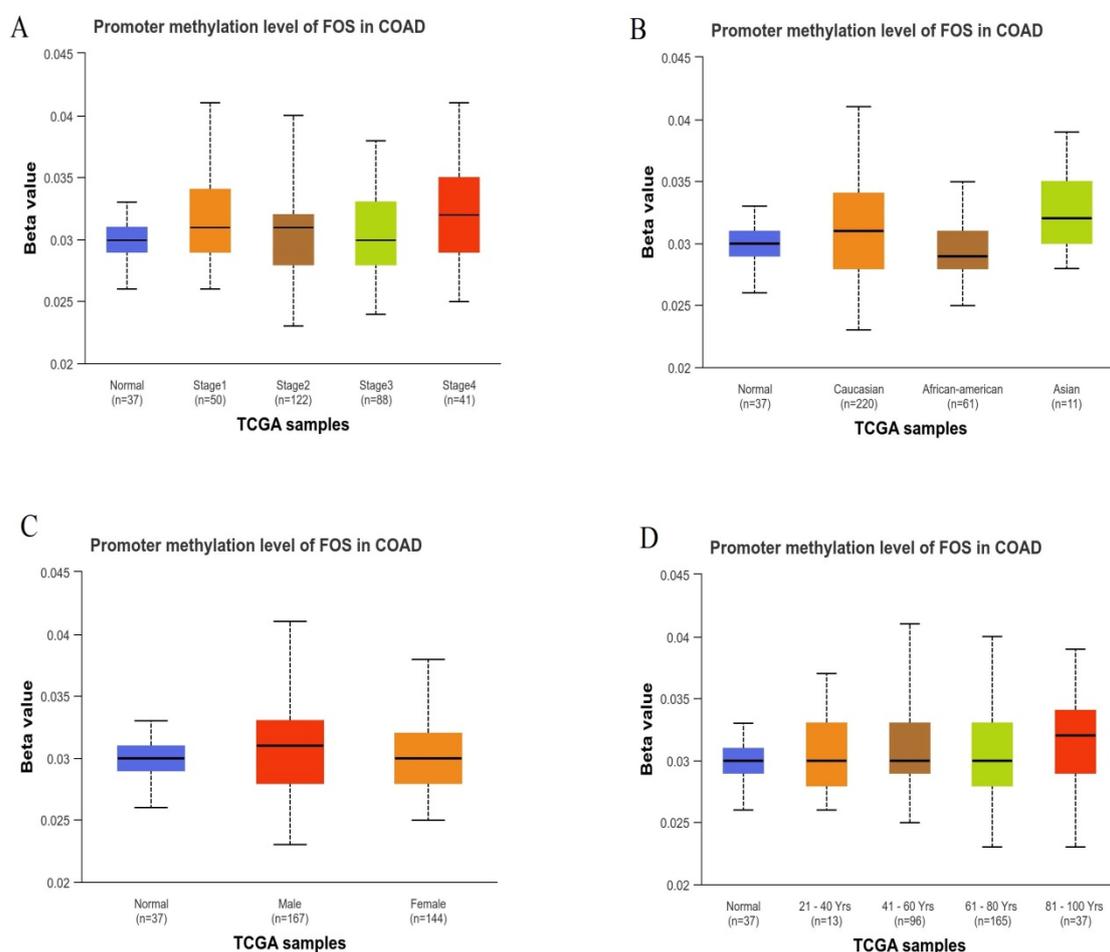
Thus, the difference in promoter methylation of FOS in COAD and normal control samples was analyzed using the UALCAN database (Figure 4). Our examination revealed significant variation, specifically hypermethylation, in the promoter methylation level of FOS in COAD compared to normal control samples. This observation suggested potential epigenetic dysregulation of FOS, highlighting its involvement in COAD pathogenesis. Such findings contribute to our understanding of the molecular mechanisms underlying COAD development and provide insights into the role of FOS as a potential biomarker or therapeutic target in COAD management.



**Figure 4:** Promoter methylation pattern of FOS in COAD and normal control samples

### Promoter methylation of FOS in COAD samples divided based on different clinical parameters

To further investigate the promoter methylation of FOS in COAD, we examined various clinical parameters (Figure 5). Primarily, we explored FOS promoter methylation across different COAD stages compared to normal control samples. Significant variations were observed among stages, with all four stages exhibiting noticeable hypermethylation (Figure 5A). Subsequently, we analyzed FOS promoter methylation considering the race of COAD patients. We found that hypermethylation occurred in FOS promoter regions across all racial groups compared to normal control samples (Figure 5B). Following this, assessment of FOS promoter methylation according to patient gender revealed gender-specific variations, with both females and males exhibiting hypermethylation (Figure 5C). Finally, we investigated FOS promoter methylation with respect to patient age, uncovering varying methylation levels across different age groups (Figure 5D). These comprehensive analyses highlight the intricate association between FOS promoter methylation and various clinical parameters in COAD, providing insights into the diverse mechanisms underlying FOS expression regulation in COAD pathogenesis.

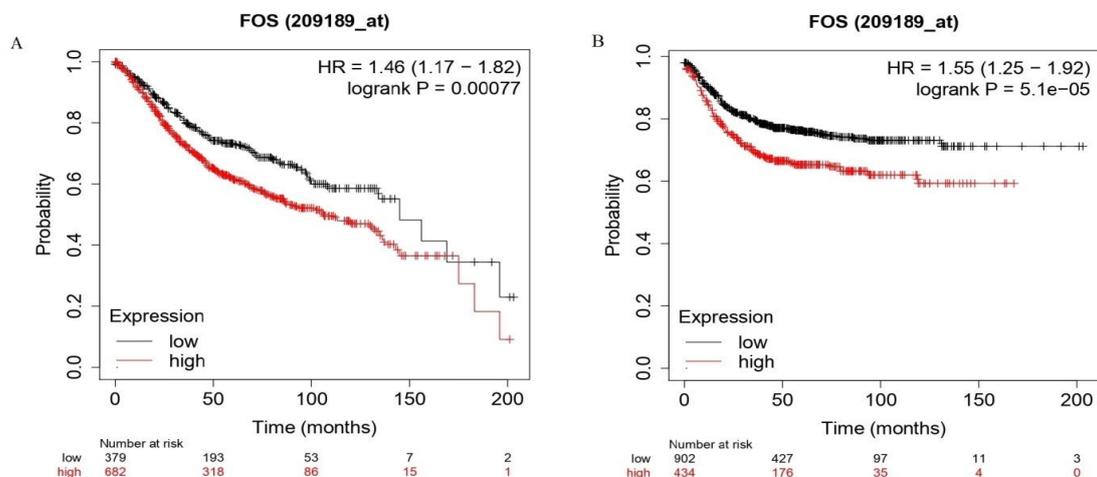


**Figure 5:** FOS promoter methylation pattern across different clinical parameters

### Survival analysis of FOS

To further evaluate FOS gene expression in COAD, we conducted an investigation for overall survival (OS) and disease-free survival (DFS) using the KM plotter tool. The current study revealed a significant association between FOS gene expression and patient survival outcomes. Specifically, COAD patients with low FOS expression experienced shorter overall survival compared to those with high FOS expression levels (Figure 6A). Similarly, in the disease-free survival (DFS) analysis, COAD patients with higher FOS expression experienced shorter DFS compared to COAD patients with low FOS expression. These findings underscore the crucial role of FOS in influencing the

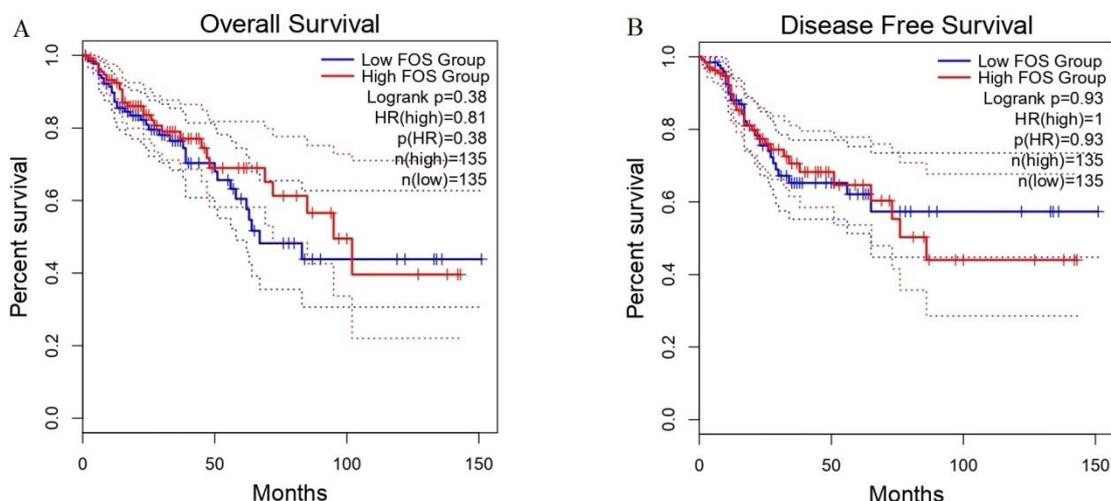
survival outcomes of COAD patients, highlighting its potential clinical significance as a prognostic marker in COAD management.



**Figure 6: KM survival curve (OS, RFS) of FOS in COAD patients**

### Prognostic analysis of FOS in COAD

The GEPIA2.0 dataset was utilized to assess the prognostic value of FOS expression in COAD cancer. We divided COAD patients into low and high expression groups based on FOS expression levels. In COAD, high FOS expression was associated with better overall survival (OS) compared to low FOS expression (Figure 7A). Additionally, we found that low FOS expression levels were associated with favorable disease-free survival (DFS) in COAD compared to the high FOS expression group (Figure 7B).



**Figure 7: Survival curve (OS, RFS) of FOS in COAD patients**

### Mutational analysis of FOS in COAD cancer

To investigate the mutation features of FOS, we conducted a comprehensive mutational analysis of FOS in COAD cancer using the cBioPortal database. In the current review, no significant mutations of FOS were observed (Figure 8).



**Figure 8: Oncoplot of FOS in COAD cancer**

## Discussion

In this review, we conducted a comprehensive investigation of FOS expression, prognosis, methylation, survival, and mutation in COAD using various bioinformatics online tools. Furthermore, overall survival (OS) and disease-free survival (DFS) were employed to validate the significance of differentially expressed genes in colon adenocarcinoma. Our findings suggest that FOS plays a critical role in human physiology and indicate a potential connection between FOS expression and COAD proliferation, proposing FOS as a putative regulator in COAD pathogenesis [26].

The molecular mechanisms underlying colorectal cancer (CRC) remain incompletely understood by researchers. Therefore, there is a need for potential molecular signatures to elucidate the molecular mechanisms of CRC and identify therapeutic targets [27]. Unified statistics and bioinformatics analyses are now widely used to investigate possible molecular signatures of cancers [28]. Bioinformatics analysis of gene expression profiles is commonly utilized to identify differentially expressed genes (DEGs) as biomarkers for the initiation and progression of cancer [29]. Transcriptomics analysis is a popular approach for identifying DEGs between normal and cancer tissue samples [30]. Increased cell division and inhibition of apoptosis in cancer cells are widely recognized as significant markers for colon cancer [31]. The MS4A12 gene, belonging to the MS4A family, encodes a protein found in the apical membrane of colonocytes that plays a significant role in differentiation, proliferation, and cell cycle regulation, and is considered a risk classification marker for early-stage colon cancer [32]. Up-regulation of CLDN1 expression has been observed in patients with colorectal cancer, suggesting its potential as a biomarker for colorectal cancer treatment [33].

In general, the FOS gene provides instructions for making a protein called c-fos, which plays a pivotal role in regulating cell growth, division, and survival. The FOS gene family encodes leucine zipper proteins and acts as a controller of cell proliferation, differentiation, and transformation [27, 33]. Previous studies have shown that in prostate cancer cell lines, an absence of FOS promotes cell proliferation and results in changes to oncogenic pathways [34]. Additionally, past investigations have demonstrated that the AP-1 motif, which binds to c-Fos, is necessary for effective transcription of the human P53 promoter [35, 36]. Moreover, P53 is an important tumor suppressor gene that induces apoptosis [37]. In the absence of Fos, cancer volume was significantly reduced, supporting the pivotal role of Fos expression and activity in neoplastic transformation of epidermal keratinocytes [38]. Fra-1, another member of the Fos family, is over-expressed in various types of human cancers including brain, lung, esophagus, thyroid, colorectal, skin, ovary, etc. [39-45].

In the ongoing examination, UALCAN database was utilized to assess the expression of FOS in COAD. The analysis revealed down-regulated FOS expression across various stages, subtypes, age, gender, and racial groups. In terms of cancer progression, our findings showed that FOS expression levels were significantly lower in COAD tissues compared to normal control samples. Furthermore, using the KM plotter tool, our analysis revealed that COAD patients with high FOS expression experienced worse overall survival and shorter disease-free survival compared to individuals with low FOS expression levels. Our study indicates that FOS expression level in tissue serves as a poor prognostic factor independently. Further investigations are needed to explore the prognostic value of FOS expression in other cancers.

## Conclusion

The primary objective of this study was to identify key genomic biomarkers from FOS gene expression profiles for the diagnosis, prognosis, and treatment of CRC using integrated bioinformatics and statistical approaches. Additionally, promoter methylation, differential expression, and correlation analysis provided insights into potential mechanisms related to FOS in cancer. Our study underscores the need for further exploratory and clinical investigations to fully understand the potential of FOS and its practical applications in cancer therapy and prognosis prediction.

## Acknowledgement

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## Conflict of interest

None.

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