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DECIPHERING THE ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR A (VEGFA) IN COLON ADENOCARCINOMA PROGRESSION: A COMPREHENSIVE BIOINFORMATICS ANALYSIS

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Abstract
The study investigated the expression pattern and potential role of VEGFA in colon adenocarcinoma (COAD) using bioinformatics tools and databases. Initially, UALCAN analysis revealed a significant up-regulation of VEGFA expression in COAD samples compared to normal controls, indicating its involvement in COAD pathogenesis. This finding was further validated using GEPIA2, further emphasizing the overexpression of VEGFA in COAD. Subsequent analysis across different parameters such as cancer stage, patient race, gender, and age consistently showed significant overexpression of VEGFA, highlighting its potential role in COAD progression. Additionally, promoter methylation analysis demonstrated hypermethylation of VEGFA in COAD samples, suggesting its aberrant regulation in cancer development. The association between VEGFA expression and overall survival (OS) was examined using KM plotter, revealing that higher VEGFA expression is associated with poor OS in COAD patients. Furthermore, genetic alteration analysis via cBioPortal indicated a low frequency of genetic mutations in VEGFA, with amplification being

the most common mutation type. Overall, these findings suggest that VEGFA overexpression may contribute to the progression and development of COAD, emphasizing the need for further research to elucidate its precise role and potential therapeutic implications**.**

Introduction

Cancer, a formidable adversary that stealthily infiltrates the human body, poses a significant global medical and socio-economic burden, with an estimated 19 million cases reported annually since 2020 (1-5). Predictions for 2023 indicate a staggering 1.95 million new cases, accompanied by 609,802 cancer-related deaths (6). With over 200 types classified based on their location and nature within the body, cancer manifests in diverse forms (6).

Colon Adenocarcinoma (COAD), a prevalent malignant tumor worldwide, originates in gland cells responsible for mucin production, safeguarding the inner colon (7-10). Ranked as the second most fatal cancer and the third most commonly diagnosed malignancy in 2020 by the World Health Organization (WHO), COAD's incidence is alarming (10). Major risk factors contributing to its development include alcohol consumption, low-fiber and high-fat diets, sedentary lifestyles, and smoking (11, 12).

Treatment for COAD often involves targeted therapy in conjunction with chemotherapy or immunotherapy, although associated side effects such as bone marrow suppression, gastrointestinal reactions, and immune deficiency pose significant challenges (11, 12). Given the intricate relationship between genetic characteristics and therapeutic outcomes in cancer, numerous studies have underscored the importance of genetic analysis (5, 13-17). Thus, we embarked on a systematic examination of the VEGFA (vascular endothelial growth factor A) gene to delineate its prognostic implications in COAD (18, 19). VEGFA, a member of the growth factor family, plays a pivotal role in angiogenesis by promoting endothelial cell migration, proliferation, and survival, thereby enhancing vascular permeability (20-22). Overexpression of VEGFA has been associated with tumor size, proliferation, and poor survival rates, implicating its significance in cancer progression (23-25).

Despite its potential role in COAD progression, no bioinformatics studies have delved into the specific involvement of VEGFA. Therefore, this study aims to analyze VEGFA expression levels and their prognostic relevance in COAD, employing the UALCAN database, cBioPortal platform, and GEPIA 2 for comprehensive investigation.

Materials and methods

UALCAN

The analysis of TCGA cancer-related data is facilitated through the UALCAN database (26), a valuable resource for comprehensive investigation. In our study, we focused on elucidating the expression patterns of VEGFA in COAD, leveraging the capabilities of the UALCAN platform. Specifically, we examined the relationship between VEGFA expression levels and promoter methylation status, considering various parameters including sample type, patient age, gender, and race (27). By scrutinizing these factors, we aimed to gain insights into the intricate interplay between VEGFA expression dynamics and epigenetic modifications, as well as their potential associations with clinicopathological variables in COAD.

GEPIA2 (Gene Expression Profiling Interactive Analysis 2)

GEPIA2, a powerful tool designed for comprehensive expression analysis of TCGA-seq data, served as a pivotal resource in our study (28). Leveraging its capabilities, we conducted in-depth survival analysis and sample-based investigations to further elucidate the prognostic implications and sample-specific characteristics associated with VEGFA expression in COAD. By harnessing the advanced functionalities of GEPIA2, we aimed to unravel crucial insights into the survival outcomes and molecular landscape of COAD, shedding light on the clinical relevance of VEGFA expression in this context.

Kaplan-Meier Plotter

In our current study, we employed the Kaplan-Meier (KM) Plotter (29), a widely recognized web tool renowned for its ability to estimate the impact of gene expression on overall survival (OS). Utilizing this platform, we conducted an extensive analysis to evaluate the clinical significance of VEGFA in influencing the survival outcomes of patients with COAD. By leveraging the KM Plotter, we were able to ascertain hazard ratios and p-values ($p < 0.05$), providing valuable insights into the prognostic relevance of VEGFA expression in COAD patients.

cBioPortal

In our study, we utilized cBioPortal, a user-friendly database renowned for its capacity to assess cancer multi-omics data (30). Leveraging this platform, we conducted an in-depth evaluation of the genetic mutations occurring in the VEGFA gene within COAD, utilizing datasets derived from The Cancer Genome Atlas (TCGA). Through the comprehensive analysis facilitated by cBioPortal, we aimed to elucidate the genetic landscape of VEGFA alterations in COAD, providing valuable insights into the molecular mechanisms underlying this malignancy.

Results

Expression Analysis of VEGFA in COAD and normal control samples

Initially, we employed the UALCAN database to scrutinize the expression patterns of VEGFA in samples of colorectal adenocarcinoma (COAD) and their corresponding normal controls. Our analysis uncovered a notable upregulation of VEGFA expression in COAD samples when compared to the normal controls (Figure 1). This significant upregulation underscores the potential involvement of VEGFA in the proliferation of COAD. However, it is imperative to conduct further investigations to comprehensively elucidate the precise role of VEGFA in the context of COAD progression.

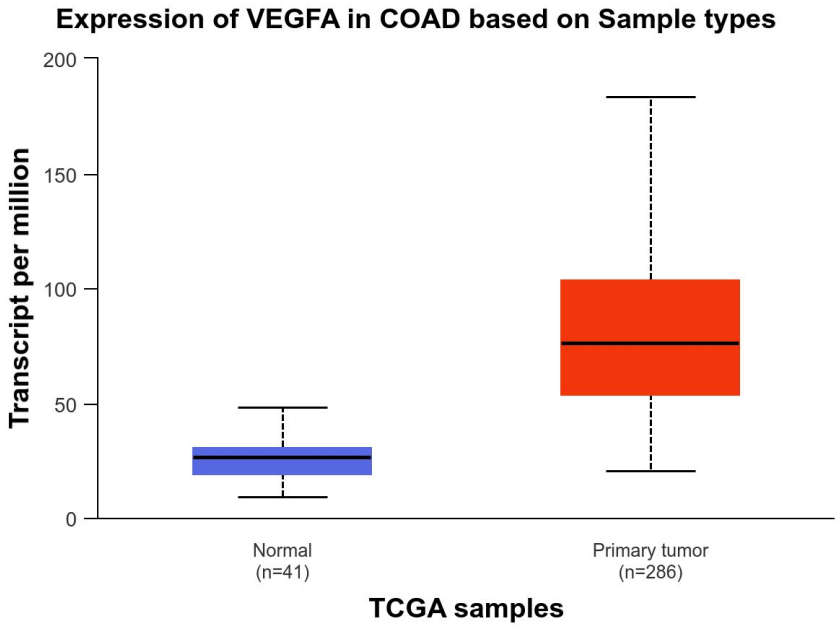

Figure 1: Expression of VEGFA in COAD and normal samples. A p-value < 0.05 was considered significant.

Validation of VEGFA expression analysis using GEPIA2

For additional validation of VEGFA expression in both COAD and normal samples, we turned to GEPIA2. Consistent with our findings from UALCAN, the analysis conducted through GEPIA2 corroborated significant overexpression of VEGFA in COAD samples compared to normal samples

(Figure 2). This alignment between the results obtained from UALCAN and GEPIA2 further strengthens the evidence supporting the pronounced upregulation of VEGFA in COAD, emphasizing its potential relevance in the context of this malignancy.

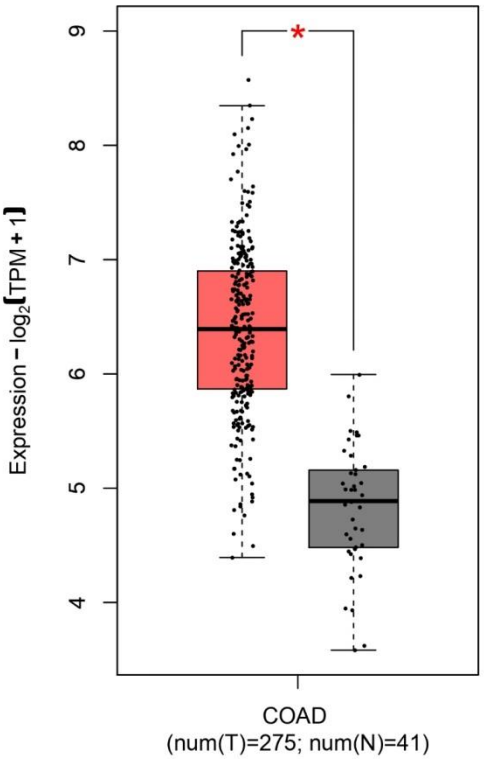

Figure 2: Validation of VEGFA expression in COAD and normal samples. A p-value < 0.05 was considered significant.

Expression analysis of VEGFA in COAD divided based on different parameters

Additionally, we conducted an analysis of VEGFA expression in COAD across various parameters. Initially, we assessed expression levels at different stages of COAD, observing a significant overexpression compared to normal samples (Figure 3A). Subsequently, we investigated VEGFA expression across different patient races, noting a significant overexpression (Figure 3B). Our exploration extended to examining the influence of patient gender on VEGFA expression in COAD, revealing significant overexpression (Figure 3C). Moreover, we analyzed the effect of patient age on VEGFA expression, finding it to be consistently overexpressed (Figure 3D). These findings underscore the role of VEGFA overexpression in COAD proliferation. However, further studies are warranted to fully elucidate these observations.

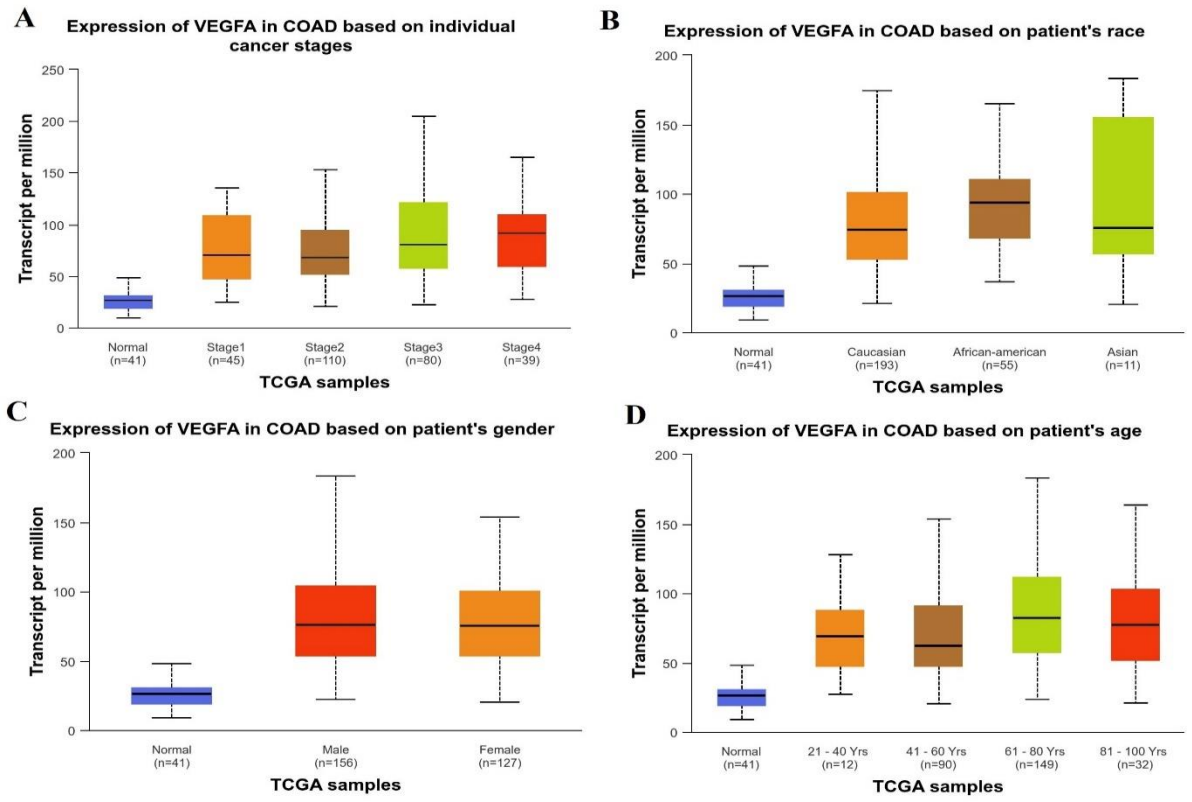

Figure 3: Expression of VEGFA in COAD patients of different clinical variables and normal samples. A p-value < 0.05 was considered significant.

Analysis of promoter methylation level of VEGFA in COAD

Prior research has highlighted the impact of irregular methylation in the promoter region of genes on cancer progression (31). Therefore, we investigated the promoter methylation levels of VEGFA in both COAD samples and normal control samples. Our findings indicated hypermethylation of VEGFA in COAD (Figure 4), suggesting a positive correlation with its expression. This aberrant behavior of VEGFA elucidates its involvement in COAD metastasis. Furthermore, we delved into the promoter methylation levels of VEGFA in COAD across various parameters such as patient age, gender, race, and individual cancer stages. Our analysis revealed significant hypermethylation of the VEGFA promoter across these parameters (Figure 5), underscoring its role in COAD proliferation. However, further investigation is imperative to deepen our understanding in this area.

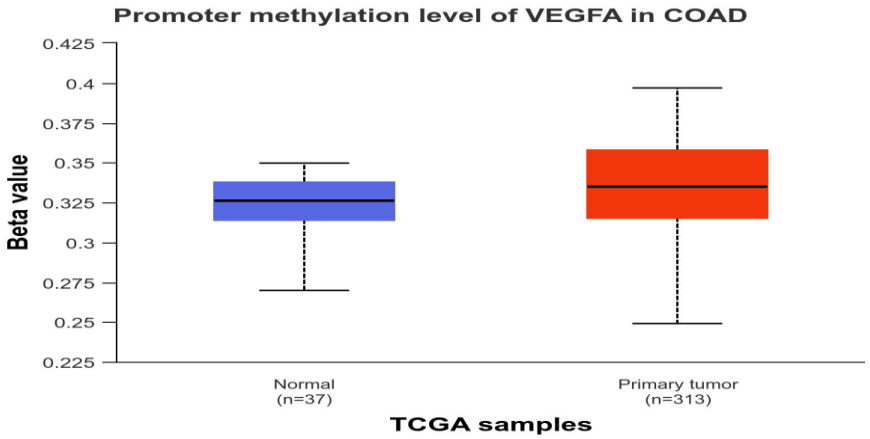

Figure 4: Promoter methylation level of VEGFA in COAD and normal samples. A p-value < 0.05 was considered significant.

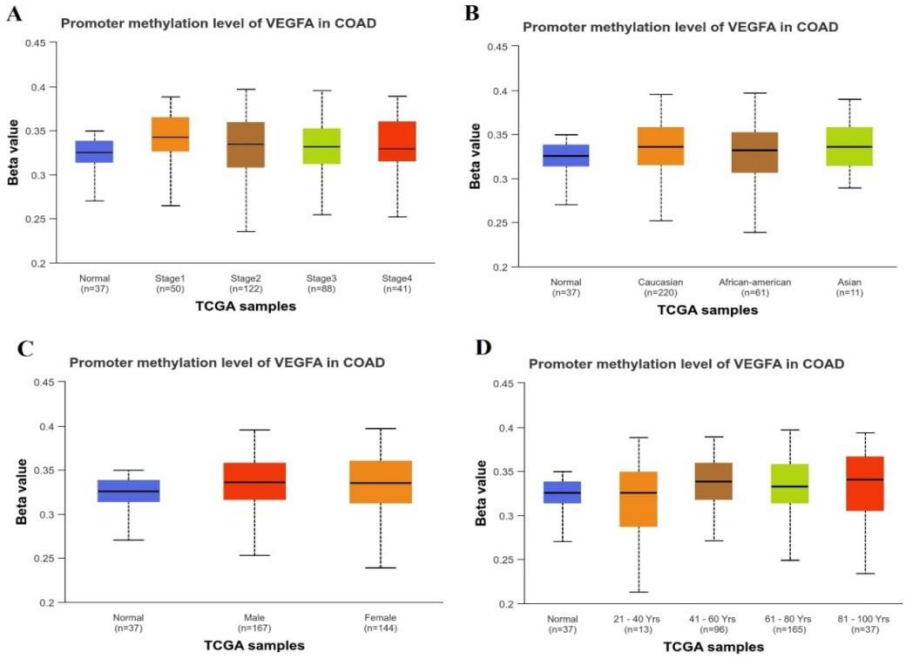

Figure 5: Promoter methylation level of VEGFA in COAD patients of different clinical variables and normal samples. A p-value < 0.05 was considered significant.

Analysis of VEGFA expression with Overall Survival

We employed KM plotter to investigate the association between VEGFA expression and overall survival (OS). Our analysis demonstrated a correlation between VEGFA overexpression and poor OS (Figure 6). Taken together, our findings strongly suggest that VEGFA plays a contributory role in the progression and development of COAD.

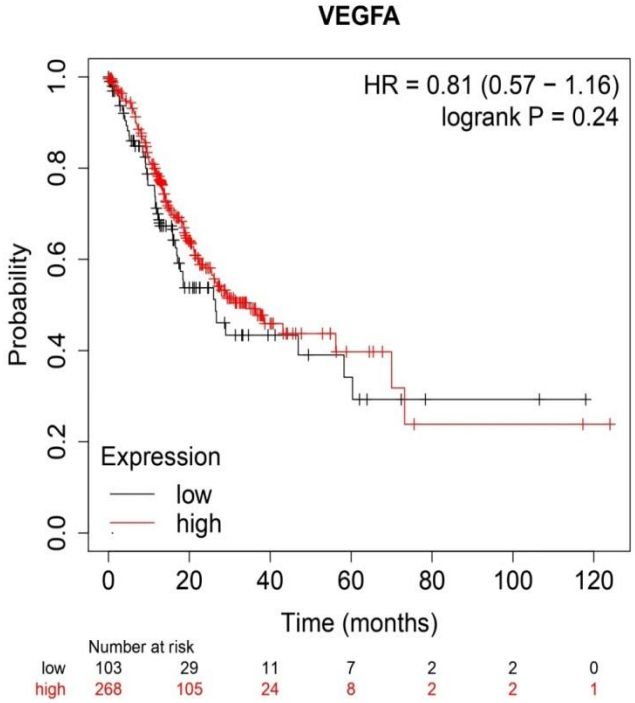

Figure 6: KM survival analysis of VEGFA in COAD patients. A p-value < 0.05 was considered significant.

Genetic alteration analysis of VEGFA

We examined the genetic mutations of VEGFA in COAD using the cBioPortal database. Our analysis revealed that only 2.3% of COAD cases exhibited genetic mutations in VEGFA (Figure 7). The most commonly observed mutation was amplification, followed by missense mutation. Overall, our findings suggest that these mutations have a relatively minor impact on COAD.

Figure 7: Mutational analysis of VEGFA in COAD patients.

Discussion

Cancer is characterized by high mortality rates, poor prognosis, and limited clinical outcomes (32-37). Thus, there is an urgent need to identify potential molecular biomarkers for the effective diagnosis and treatment of cancer without complications. Previous studies have identified VEGFA as a potential participant in tumor angiogenesis, a process crucial for tumor proliferation and metastasis (38, 39). While expression analysis of VEGFA has been extensively studied in various cancers, its specific role in Colon Adenocarcinoma (COAD) remains unclear. In this study, we aimed to investigate the expression level, promoter methylation level, prognostic value, and genetic mutations of VEGFA in COAD.

Initially, we observed a significant overexpression of VEGFA in COAD compared to normal samples. Subsequently, we explored the upregulation of VEGFA expression across various parameters such as patient age, gender, race, and individual cancer stages, consistently finding significant upregulation. To validate our findings, we utilized GEPIA2 and confirmed the correlation in our results. Previous reports have indicated that increased expression levels are typically associated with poor overall survival (OS) and tumor metastasis. Therefore, these collective findings strongly suggest that VEGFA may play a pivotal role in the progression and proliferation of COAD. Furthermore, we delved into the promoter methylation level to elucidate the expression pattern of VEGFA, as promoter methylation is known to regulate gene expression. Our analysis revealed hypermethylation of VEGFA in COAD patients compared to normal patients. Based on these results, we hypothesize that VEGFA could serve as a therapeutic target, as well as a prognostic and diagnostic biomarker in COAD.

As previous studies have elucidated, the overexpression of functional genes often serves as prognostic indicators (42). Our survival analysis further confirmed that upregulated expression of VEGFA is indeed correlated with poor overall survival (OS) in COAD patients, suggesting its potential as a prognostic indicator. Additionally, our analysis of genetic alterations using cBioPortal revealed that only 2.3% of COAD cases exhibited VEGFA genetic mutations, indicating that these mutations have minimal impact on the regulation of VEGFA. These findings align with previous research highlighting the association of VEGFA with tumor angiogenesis. Therefore, the observed overexpression, poor prognosis, and hypermethylation of VEGFA collectively explain its roles in the progression of COAD.

Conclusion

In this study, we comprehensively investigated VEGFA expression, genetic mutations, prognostic value, and promoter methylation levels, uncovering their positive associations with the development and progression of COAD. Our findings strongly suggest that VEGFA serves as both a potential therapeutic target and biomarker in COAD. However, further studies are imperative to deepen our understanding in this regard.

Acknowledgement
None
Conflict of interest
None

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