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Deciphering the Role of EPPK1 in the Development of Colon Adenocarcinoma by Integrated Bioinformatics Approach

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Abstract: The role of Epiplakin1 (EPPK1) in colon adenocarcinoma (COAD) was analyzed through a comprehensive evaluation of its expression, methylation, genetic mutations, and prognostic implications. A significant up-regulation of EPPK1 expression in COAD malignant cells compared to normal control samples was observed using data from the UALCAN database. EPPK1 expression was found to be elevated across different cancer development stages, racial groups, genders, and age groups, emphasizing its crucial role in cancer proliferation. Validation of EPPK1 expression through the GEPIA2.0 dataset further confirmed its overexpression in COAD when compared to normal samples. The analysis revealed dysregulation across all four stages of cancer development, with the highest expression in stage IV and the lowest in stage II. Additionally, promoter methylation analysis demonstrated a fundamental relationship between COAD samples and normal controls, revealing significant methylation patterns across different clinical parameters, including cancer stages, race, gender, and age. Overall survival (OS) and disease-free survival (DFS) analysis using the KM plotter showed a strong association between high EPPK1 expression and worse survival outcomes in COAD patients. Conversely, low EPPK1 expression was linked to better OS and DFS. Genetic mutation analysis using cBioPortal identified minimal EPPK1 mutations in COAD, predominantly truncating and missense mutations, highlighting their relevance to EPPK1 dysregulation in COAD. These findings underscore the important role of EPPK1 in COAD development and proliferation, suggesting its potential as a therapeutic target and prognostic marker. Further exploration of EPPK1's molecular mechanisms and its involvement in the COAD microenvironment may reveal new pathways for targeted treatments and precision medicine strategies against this challenging disease.

Keywords: EPPK1; Colon adenocarcinoma; Biomarker; Prognosis

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1. Introduction

Colon adenocarcinoma (COAD) is one of the most common malignant tumors [1,2]. According to data released by

the World Health Organization's International Agency for Research on Cancer (IARC) in 2020, COAD ranks as the third most common cancer and the second leading cause of cancer-related deaths worldwide [3]. Approximately 900,000 patients die each year from COAD, largely due to its late clinical diagnosis [4]. Moreover, the incidence and mortality rates of COAD continue to rise, partly due to the poor prognosis of advanced cases. Recurrence and metastasis are significant factors contributing to the poor outcomes of COAD. The 5-year and 10-year survival rates for patients with metastatic COAD are 40% and 20%, respectively [5]. Treatment decisions are mainly guided by the tumor node metastasis (TNM) staging system [6]. COAD is a heterogeneous disease with genetic and clinicopathologic features driving its occurrence and progression [7]. Most COAD patients are diagnosed with resectable tumors and treated with surgery, often followed by adjuvant therapy if necessary. For advanced colorectal cancer, targeted therapy combined with chemotherapy (using oxaliplatin or irinotecan) is the standard treatment. However, current first-line chemotherapy regimens are often associated with severe side effects, including gastrointestinal reactions, immune system damage, and bone marrow suppression [4]. Chemotherapeutic agents are non-specific and cytotoxic, affecting any normal, dividing cell in the body. Immunotherapy has emerged as a promising alternative for COAD treatment, with immune checkpoint therapy receiving regulatory approval in 2017. This therapy primarily benefits COAD patients with high microsatellite instability (MSI-H) or deficient mismatch repair (dMMR) [8].

Epiplakin1 (EPPK1), a member of the plakin family, has been identified as a human epidermal self-antigen, commonly expressed in the esophagus and other organs [9,10]. As a cytolinker protein, EPPK1 is located at the junction of the cytoplasmic layers and coordinates the cytoskeleton with myofilaments [11]. EPPK1, encoded on chromosome 8q24.3, has a molecular mass of 450 kDa and plays a role in linking intermediate filaments and regulating their reorganization in response to stress [12-14]. Alterations in the *EPPK1* gene have been associated with poor prognosis in early-stage lung adenocarcinoma [15]. Previous studies have also suggested that down-regulation or absence of EPPK1 promotes cell migration and proliferation in the human corneal epithelium [16]. While the precise function of EPPK1 remains unclear, evidence suggests that EPPK1 may play a significant role in cancer development. Recent studies have shown that EPPK1 is involved in the progression of various cancers, including liver cancer [17], cervical cancer [18], and bladder urothelial carcinoma [19].

This study aims to investigate the alterations, expression levels, prognostic impact, and functional significance of EPPK1 in the context of COAD using bioinformatics analysis. In addition, the relationship between *EPPK1* expression and promoter methylation levels is explored. Data from multiple sources, including The Cancer Genome Atlas (TCGA), UALCAN, Kaplan-Meier, GEPIA2.0, and cBioPortal, were utilized. The primary objective of this study is to assess the expression pattern of the *EPPK1* gene in COAD and to understand its potential implications for cancer treatment and prognosis.

2. Materials and methods

2.1. Prognostic analysis

Gene Expression Profiling Interactive Analysis (GEPIA) is a widely used and highly referenced resource for gene expression analysis, based on cancer and normal samples from the TCGA and GTEx datasets [20]. GEPIA2.0 extends gene expression quantification from the gene level to the transcript level and supports the analysis of specific cancer subtypes and correlations between subtypes. Additionally, GEPIA2.0 incorporates new techniques for gene signature quantification, inspired by single-cell sequencing studies, and provides users with a customizable platform to upload their own RNA-seq data for comparison with TCGA and GTEx samples.

2.2. Expression analysis

UALCAN, a user-friendly and freely accessible online tool, was utilized in this study to analyze TCGA genomics data [21]. Specifically, UALCAN was used to investigate *EPPK1* expression levels and promoter methylation status in COAD. UALCAN also enabled the evaluation of *EPPK1* expression and promoter methylation across various clinical parameters, including patient race, age, and gender. This comprehensive analysis provided valuable insights into the association between *EPPK1* expression patterns, promoter methylation, and demographic factors in COAD patients.

2.3. Survival analysis

The Kaplan-Meier plotter is widely recognized for survival analysis in cancer research [22]. This tool uses resources from TCGA to retrieve information on mRNA expression levels. Its user-friendly interface allows researchers to easily assess the prognostic value of specific genes. In this study, the Kaplan-Meier plotter was employed to examine the impact of *EPPK1* expression on overall survival (OS) in patients with COAD. This analysis provided important insights into the potential prognostic significance of *EPPK1* in COAD.

2.4. Mutational analysis

cBioPortal, a widely used and essential database for cancer genomics research [23], offers extensive data on copy number variations, genetic mutations, and other genomic alterations, drawing from a dataset of over 28,000 samples. In this study, cBioPortal was used to investigate *EPPK1* mutations specifically in COAD, utilizing the comprehensive data available from TCGA. This analysis offered valuable insights into the prevalence and characteristics of *EPPK1* mutations in COAD patients, contributing to the understanding of the molecular patterns associated with this cancer type.

3. Results

3.1. Expression analysis of EPPK1 in COAD

Using the UALCAN dataset, the expression of *EPPK1* was analyzed across both normal and malignant tissues (**Figure 1**). The analysis revealed a significant up-regulation of *EPPK1* in COAD cancer compared to normal control samples, suggesting a potential association between *EPPK1* expression and the proliferation of COAD cancer cells.

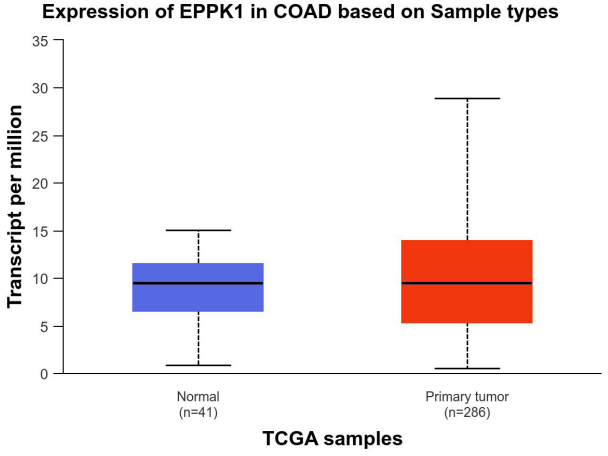

Figure 1. Expression profiling of *EPPK1* in COAD and normal tissue samples

3.2. Expression analysis of EPPK1 in COAD based on different clinical parameters

EPPK1 expression in COAD samples was further analyzed across various clinical parameters, including cancer stages, race, gender, and age (**Figure 2**). Initially, the analysis showed a consistent up-regulation of *EPPK1* in COAD across all stages except stage II, where a noticeable down-regulation was observed compared to normal controls (**Figure 2A**). *EPPK1* expression was also up-regulated in Caucasian and African-American patients, while down-regulation was observed in Asian patients (**Figure 2B**). Additionally, both male and female COAD patients exhibited a significant up-regulation of *EPPK1* compared to normal controls (**Figure 2C**). Lastly, *EPPK1* expression was up-regulated across all age groups in COAD patients (**Figure 2D**).

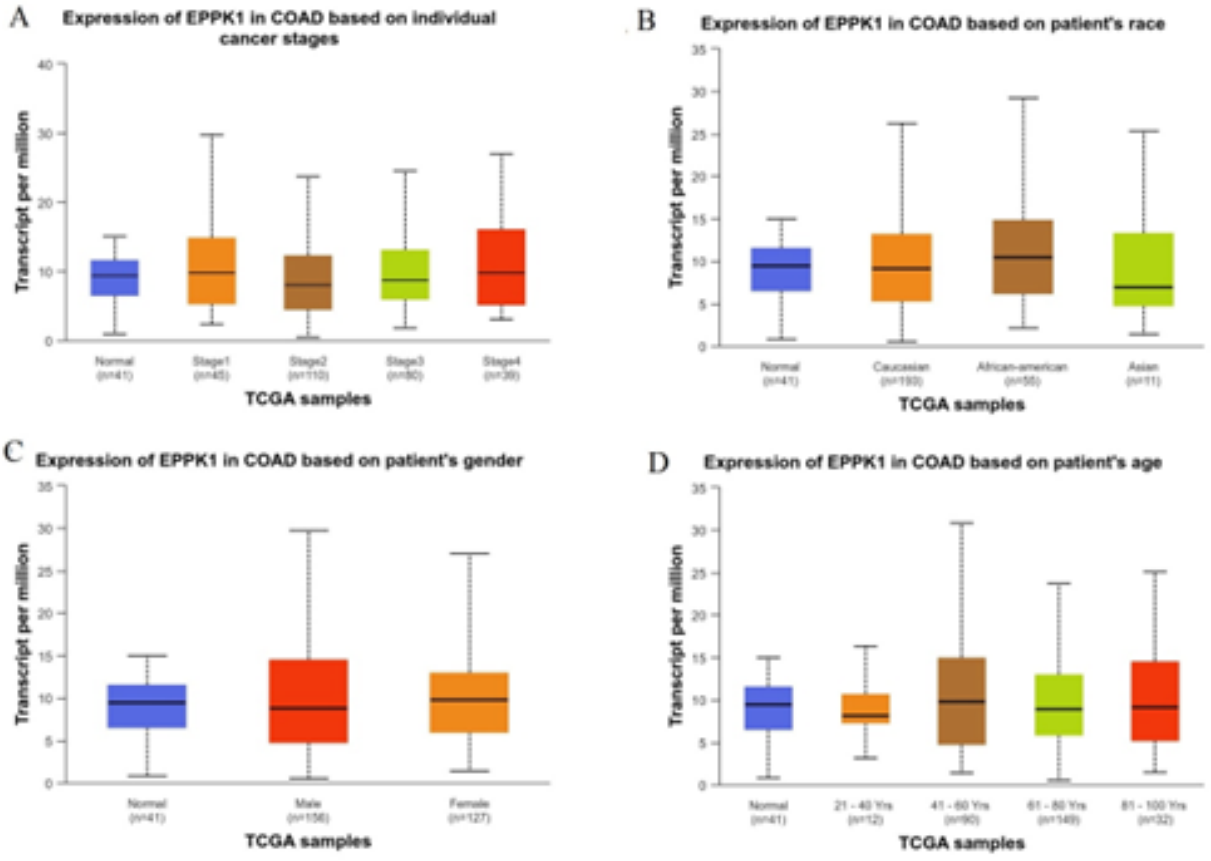

Figure 2. Expression profiling of *EPPK1* across different clinical parameters

3.3. Prognosis of EPPK1 in COAD

To further validate *EPPK1* expression in COAD, the GEPIA2.0 tool was used, confirming high *EPPK1* expression in COAD compared to normal control samples (**Figure 3A**). *EPPK1* expression was also significantly associated with different pathological stages, showing the highest expression in stage IV and the lowest in stage II (**Figure 3B**).

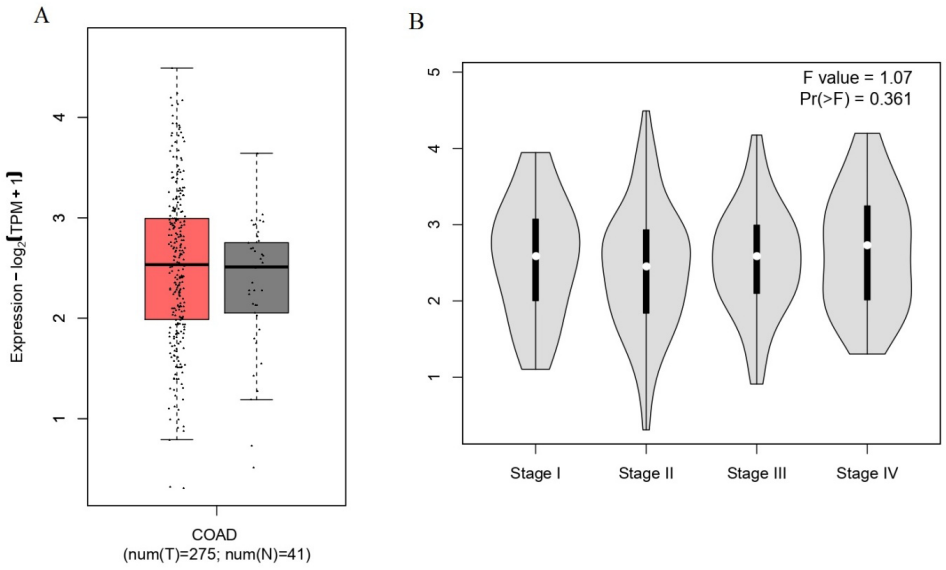

Figure 3. Validation of *EPPK1* across different stages of COAD

3.4. Promoter methylation in COAD and normal control samples

Promoter methylation of *EPPK1* in COAD and normal control samples was examined using UALCAN (**Figure 4**). The analysis revealed substantial hypermethylation of the *EPPK1* promoter in COAD compared to normal controls, suggesting potential epigenetic dysregulation of *EPPK1* in COAD pathogenesis. These findings provide insights into the molecular mechanisms underlying COAD development and emphasize *EPPK1*'s role as a potential biomarker or therapeutic target.

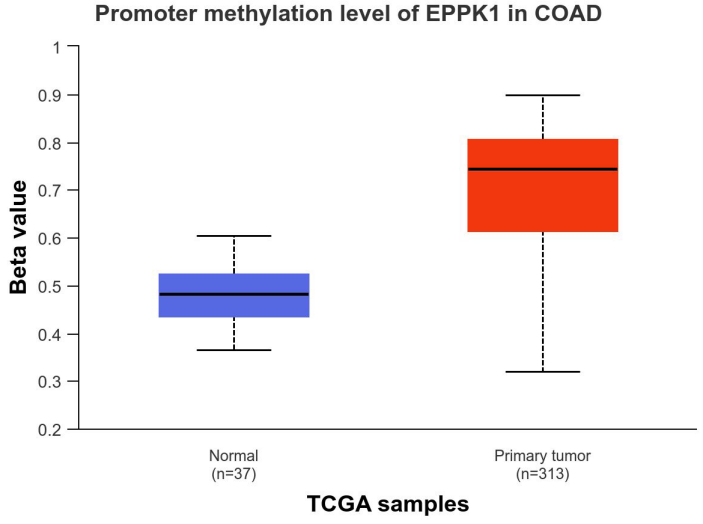

Figure 4. Promoter methylation pattern of *EPPK1* in COAD and normal control samples

3.5. Promoter methylation of EPPK1 in COAD based on different clinical parameters

Promoter methylation of *EPPK1* was further analyzed based on various clinical parameters (**Figure 5**). The analysis revealed significant hypermethylation across all COAD stages compared to normal controls (**Figure**

5A). Similar hypermethylation patterns were observed across all racial groups (**Figure 5B**), as well as among both male and female patients (**Figure 5C**). Promoter methylation levels also varied across different age groups (**Figure 5D**). These analyses highlight the strong association between *EPPK1* promoter methylation and various clinical factors in COAD.

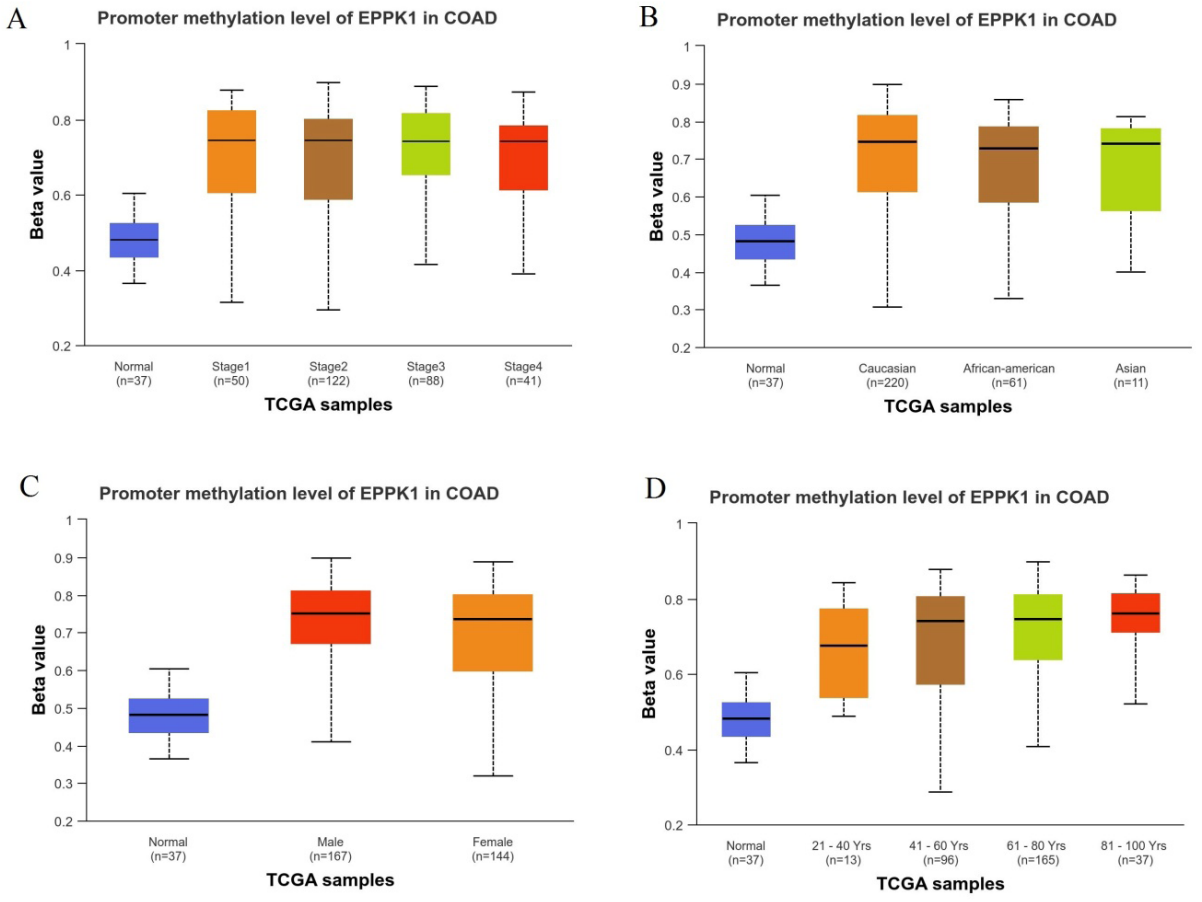

Figure 5. *EPPK1* promoter methylation pattern across different clinical parameters

3.6. Survival analysis of EPPK1

Survival analysis was conducted using the KM plotter tool to assess OS and disease-free survival (DFS) in COAD patients. The results indicated that COAD patients with low *EPPK1* expression had better OS compared to those with high *EPPK1* expression (**Figure 6A**). In contrast, patients with low *EPPK1* expression experienced worse DFS compared to those with high *EPPK1* expression (**Figure 6B**). These findings underscore the critical role of *EPPK1* in influencing COAD patient survival outcomes, highlighting its clinical significance as a prognostic marker.

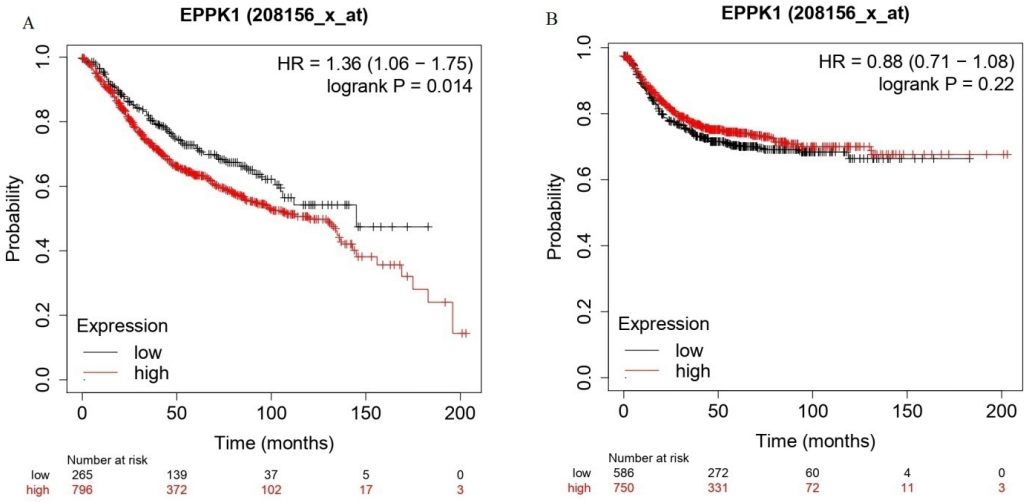

Figure 6. KM survival curves (OS and DFS) of *EPPK1* in COAD patients

3.7. Prognostic analysis of EPPK1 in COAD

The GEPIA2.0 database was also used to explore the prognostic significance of *EPPK1* expression in COAD. COAD patients were divided into low and high-expression groups. High *EPPK1* expression was associated with better overall survival compared to low expression (**Figure 7A**). Similarly, low *EPPK1* expression was linked to shorter DFS compared to high expression levels (**Figure 7B**).

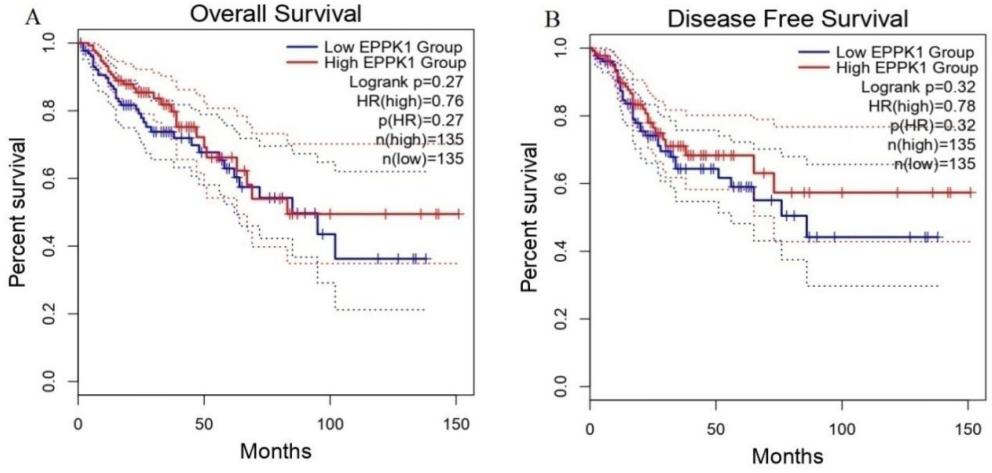

Figure 7. Survival curves (OS and DFS) of *EPPK1* in COAD patients

3.8. Mutation analysis of EPPK1 in COAD

To investigate *EPPK1* mutations in COAD, a mutational analysis was performed using the cBioPortal dataset. No significant genetic alterations in *EPPK1* were observed in this analysis (**Figure 8**).

Figure 8. Oncoplot of *EPPK1* in COAD

4. Discussion

This investigation examined *EPPK1* expression, diagnosis, methylation, survival, and mutation in COAD using various bioinformatics tools. Additionally, OS and DFS analyses were conducted to validate differentially expressed markers in COAD. The results suggest that *EPPK1* significantly influences the progression of COAD, indicating a potential association between *EPPK1* expression and the expansion of COAD, and proposing *EPPK1* as a potential regulator in COAD pathogenesis.

Colorectal cancer is the third most lethal cancer, causing approximately 700,000 deaths worldwide annually [24]. COAD remains one of the most common cancers globally, although the effectiveness of current systemic treatment options is still limited [2,4]. At the time of diagnosis, approximately 20%–25% of COAD patients present with metastatic disease, and 25% develop locally recurrent or metastatic disease within five years. The five-year survival rate for patients with metastatic COAD is only 15% [25]. It is therefore essential to investigate novel therapeutic targets to enhance treatment strategies, as current immunotherapy approaches are not effective for all cancer patients. Surgical resection is the primary treatment option, supplemented by 5-fluorouracil chemotherapy. Recently, the five-year survival rate of COAD patients has increased due to the use of various immunotherapies as alternative treatments [26,27]. Previous studies have shown that inhibitors targeting PD-1, PD-L1, and CTLA4 can affect refractory (MSI-H and MSS) colorectal tumors [28,29]. However, patient recurrence and adverse reactions have been reported. Consequently, identifying significant immune targets in COAD may improve immunotherapy outcomes.

Members of the plakin family, which are abundant in various organs and tissues, contain a conserved domain known as the plakin domain [30]. However, the specific expression levels of plakins in different species and organs remain unclear. As a member of the plakin family, *EPPK1* has been found to be expressed in various tissues and cells, particularly in pancreatic tumors [31,32], suggesting that *EPPK1* may act as a potent regulator of cancer progression. Smoking is a well-known risk factor for lung cancer [33] due to its capacity to cause DNA damage, as indicated by the presence of γH2AX [34], and to induce genomic instability, which contributes to tumorigenesis [35]. Previous research has shown that smoking can impact genomic features associated with tumorigenesis [36]. Smoking up-regulates *EPPK1* expression in normal bronchial epithelial cells, and high *EPPK1* expression is correlated with smoking exposure (pack-years) and the early stages of lung adenocarcinoma. It is hypothesized that smoking-induced DNA damage and genomic instability contribute to increased *EPPK1* expression in normal bronchial epithelial cells, potentially promoting lung cancer development. Previous studies have also linked *EPPK1* to poor prognosis in hepatocellular carcinoma and esophageal squamous cell carcinoma [37], as well as its potential as a biomarker for pancreatic and cervical cancer [32]. However, the critical role of *EPPK1* in lung cancer remains unexplored. *EPPK1* is believed to play a pivotal role in lung cancer progression via the EMT signaling pathway, which is associated with tumorigenesis [38]. *EPPK1* knockout has been shown to result in the down-regulation of MYC and the up-regulation of p53 expression at both the protein and RNA levels. MYC and p53 regulate each other and can lead to cell cycle arrest in the G1 or G2 phase, which represents an early event in tumorigenesis due to genomic instability [39,40]. However, the role of *EPPK1* in COAD remains to be further investigated.

In this study, the UALCAN dataset was employed to determine the expression of *EPPK1* in COAD. The analysis showed that *EPPK1* expression was up-regulated across various cancer stages, individual cancer types, age groups, genders, and racial groups. Regarding disease progression, the results demonstrated that *EPPK1* expression was significantly higher in COAD tissues compared to normal control samples. Additionally,

analysis using the KM plotter tool revealed that COAD patients with high *EPPK1* expression experienced shorter OS, while those with low *EPPK1* expression had shorter DFS. These findings indicate that *EPPK1* expression in tissue serves as an independent poor prognostic factor. Further studies are needed to explore the prognostic significance of *EPPK1* expression in cancer development.

5. Conclusion

In conclusion, the analysis shows that *EPPK1* overexpression in COAD is strongly associated with poor OS, promoter methylation levels, and genetic alterations. By utilizing various public datasets, including UALCAN, TCGA, cBioPortal, GEPIA2.0, and KM plotter, insights were gained into the diagnostic, prognostic, and potential therapeutic roles of *EPPK1* in COAD. However, further studies are warranted to validate and confirm these findings and to clarify the underlying mechanisms driving *EPPK1* dysregulation in COAD. These insights could ultimately contribute to the development of advanced diagnostic tools and therapeutic strategies for COAD patients.

Disclosure statement

The authors declare no conflict of interest.

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