Article

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Upregulated AGMAT is Associated with Prognosis in Colorectal Cancer

Hongyun Li[1,†], Yang Xu[1,†], Bo He[2], Dongdong Ru[3,*]

[1]Department of Medical Oncology, The Third Medical Center of Chinese PLA General Hospital, 100039 Beijing, China
[2]Department of Oncology, Fifth Medical Center, PLA General Hospital, 100039 Beijing, China
[3]Department of General Surgery Three Ward, Jincheng People's Hospital, 048026 Jincheng, Shanxi, China
*Correspondence: drrudongdong@hotmail.com (Dongdong Ru)
[†]These authors contributed equally.
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Background: Agmatinase (AGMAT) has been specifically reported to be highly expressed in several malignancies. Therefore, this study aims to investigate the role of AGMAT in colorectal cancer (CRC) and assess its potential as a prognostic biomarker.
Methods: We used The Cancer Genome Atlas (TCGA) database to identify differentially expressed genes in CRC compared to the healthy controls. Subsequently, we performed enrichment analyses using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) databases to gain insights into the biological processes and cellular signaling pathways influenced by these differentially expressed genes (DEGs). The most informative prognostic genes among these DEGs were predicted by combining Least Absolute Shrinkage and Selection Operator (LASSO) regression and best subset selection, ultimately identifying the eight most relevant genes. Furthermore, we developed gene-based risk scores using Cox coefficients for these eight prognostic genes. The prognostic ability of the risk score was evaluated using two methods: time-dependent receiver operating characteristic (ROC) analysis and Kaplan-Meier (KM) survival analysis. Additionally, the correlation between AGMAT expression and prognosis in CRC tissues was investigated utilizing GEPIA 2 and the Human Protein Atlas (HPA) database. Finally, we employed RT-qPCR to quantify *AGMAT* mRNA expression levels in CRC tissues samples.
Results: Our analysis of TCGA data revealed 1917 DEGs, including 928 upregulated and 989 downregulated genes. Among them, 8 genes, including Matrix Metallopeptidase 1 (*MMP1*), Serine Protease Inhibitor Kazal-Type 1 (*SPINK1*), Cluster of Differentiation 24 (*CD24*), Serine Protease Inhibitor Kazal-Type 4 (*SPINK4*), *AGMAT*, EPH Receptor B2 (*EPHB2*), C2 Calcium-Dependent Domain Containing 4A (*C2CD4A*), Pituitary Tumor-Transforming Gene 1 (*PTTG1*), were significantly associated with prognosis in CRC. Furthermore, we observed upregulated AGMAT expression in CRC tissues. Additionally, we found a significant association between elevated AGMAT expression and decreased overall survival (OS) and disease-free survival (DFS) in patients with CRC (p-value < 0.05).
Conclusions: This study demonstrates that AGMAT is highly expressed in CRC tissues and is significantly associated with poorer prognosis in CRC patients. These findings suggest that AGMAT expression could serve as a biomarker for predicting survival in CRC, potentially offering new insights into its role in CRC development and paving the way for future treatment options.

Keywords: agmatinase; differently expressed genes; colorectal cancer; prognosis

Introduction

Colorectal cancer (CRC) remains a widespread malignancy affecting the gastrointestinal tract [1]. Currently, it ranks third in global incidence and second in mortality rate [2]. Since CRC primarily occurs in the colon and rectum, it is classified into colon and rectal cancers based on the site of origin [3]. As these two tumors share histological features and pathogenesis, these two types of tumors are generally grouped as colorectal cancer for research purposes. It is estimated that by 2035, there could be 25 million new cases of colorectal cancer globally [4]. An epidemiological study shows a close correlation between gender, age and the occurrence of CRC [5]. Genetic and environmental components play a significant role in the development of colorectal cancer [6]. Although the genome-wide study has successfully identified susceptibility genes (single nucleotide polymorphisms) associated with colorectal cancer, the exact genetic factors remain unclear and need further study [7]. Currently, surgery is the primary treatment strategy for CRC [8]. However, most colorectal cancers are detected at advanced stages, or metastases occur in most cases, thus, additional therapies like chemotherapy and radiotherapy are essential to reduce the risk of recurrence and improve the prognosis of patients [9,10]. The lack of ideal tumor markers often hampers the timely diagnosis and treatment of colorectal cancer.

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Nevertheless, the existing known tumor markers generally possess low sensitivity and fail to meet the clinical needs for early tumor prediction [11]. Hence, it is essential to identify new tumor markers capable of facilitating early diagnosis and prediction of prognosis. Nowadays, microarray technology and bioinformatics analysis have been widely used to screen disease biomarkers, making significant contributions to the exploration of disease genes and molecular mechanisms [12–15]. Recent studies have identified crucial genes and their associated pathways in CRC through bioinformatics analysis [16–18]. In this study, the role of agmatinase (AGMAT) in CRC was explored through bioinformatics analysis utilizing The Cancer Genome Atlas (TCGA) database and the integration of clinical data.

Materials and Methods

Data Collection and Analysis

We investigated gene expression changes in colorectal cancer (CRC) using data from The Cancer Genome Atlas (TCGA) database (https://www.cancer.gov/ccg/research/genome-sequencing/tcga). This dataset included clinical information and sequencing results of 620 CRC tumor cases. Furthermore, we analyzed differential expression using the Limma package (version 3.40.2, Walter and Eliza Hall Institute of Medical Research (WEHI), Parkville, Victoria, Australia) within the R environment to identify genes with significantly altered expression. However, false discoveries were minimized by adjusting *p*-values obtained from TCGA data. Hence, genes with a fold change (log2 scale) greater than or equal to 2 and an adjusted *p*-value less than 0.05 were designated as differentially expressed genes (DEGs).

Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) Enrichment Analysis

DEGs were profiled through functional enrichment analysis to confirm their biological functions [19]. The biological roles of these identified genes were assessed using Gene Ontology (GO) enrichment analysis. The GO examines genes across three categories: biological process (BP), cellular component (CC), and molecular function (MF). Furthermore, we employed the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis to identify enriched biological pathways associated with these genes. The ClusterProfiler package in R software (Bioinformatics Group, Laboratory of Cancer Systems Biology (LCSB), Luxembourg City, Luxembourg) was utilized to analyze the GO functions of potential targets and enrich KEGG pathways. Additionally, the ggplot2 package was employed to plot boxplots, and the pheatmap package was used for heat mapping.

Signature Model Analysis

The top 600 DEGs with the most significant differences were collected for subsequent prognostic model analysis [20]. The differences in survival rates between patients with varying gene expressions were assessed using the log-rank test. Additionally, the predictive accuracy of these genes and the resulting risk scores were evaluated by the time receiver operating characteristic (ROC) analysis.

We used the glmnet package in R for feature selection through Least Absolute Shrinkage and Selection Operator (LASSO) regression. To identify the optimal model complexity, we applied a 10-fold cross-validation approach.

Utilizing the R package SURVIVE, we performed multivariate Cox regression analysis to construct prognostic models, aiming to assess the influence of multiple variables on survival outcomes among CRC patients.

GEPIA 2 Database Analysis

The GEPIA 2 database (http://gepia2.cancer-pku.cn/#index) was used to get the expression, disease-free survival (DFS), and overall survival (OS) of AGMAT among CRC patients.

Human Protein Atlas (HPA) Database Analysis

We utilized the HPA database (https://www.proteinatlas.org/) to compare AGMAT protein expression levels between normal colorectal tissues and the CRC patient's tissues.

Clinical Samples

A total of 78 CRC patients, who underwent surgeries at the General Hospital of the Chinese People's Liberation Army from December 2020 to June 2022 were included in this study. However, their paracancerous tissues were collected as the control sample. Furthermore, the study design adhered to the Declaration of Helsinki and the ethics committee of Jincheng People's Hospital approved this study (Approval number: JC-2021-35).

Inclusion and Exclusion Criteria

Inclusion Criteria: The patients who met the following criteria were included in this study: (1) The pathologically diagnosed CRC patients, (2) those aged 18–65 years, (3) the patients who were treatment-naive before surgery and have not received any specific interventions, (4) those with an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2 [21], (5) patients who provided adequate tissue samples (from surgery) for subsequent analysis, (6) and patients who submitted written informed consent before their inclusion in the study.

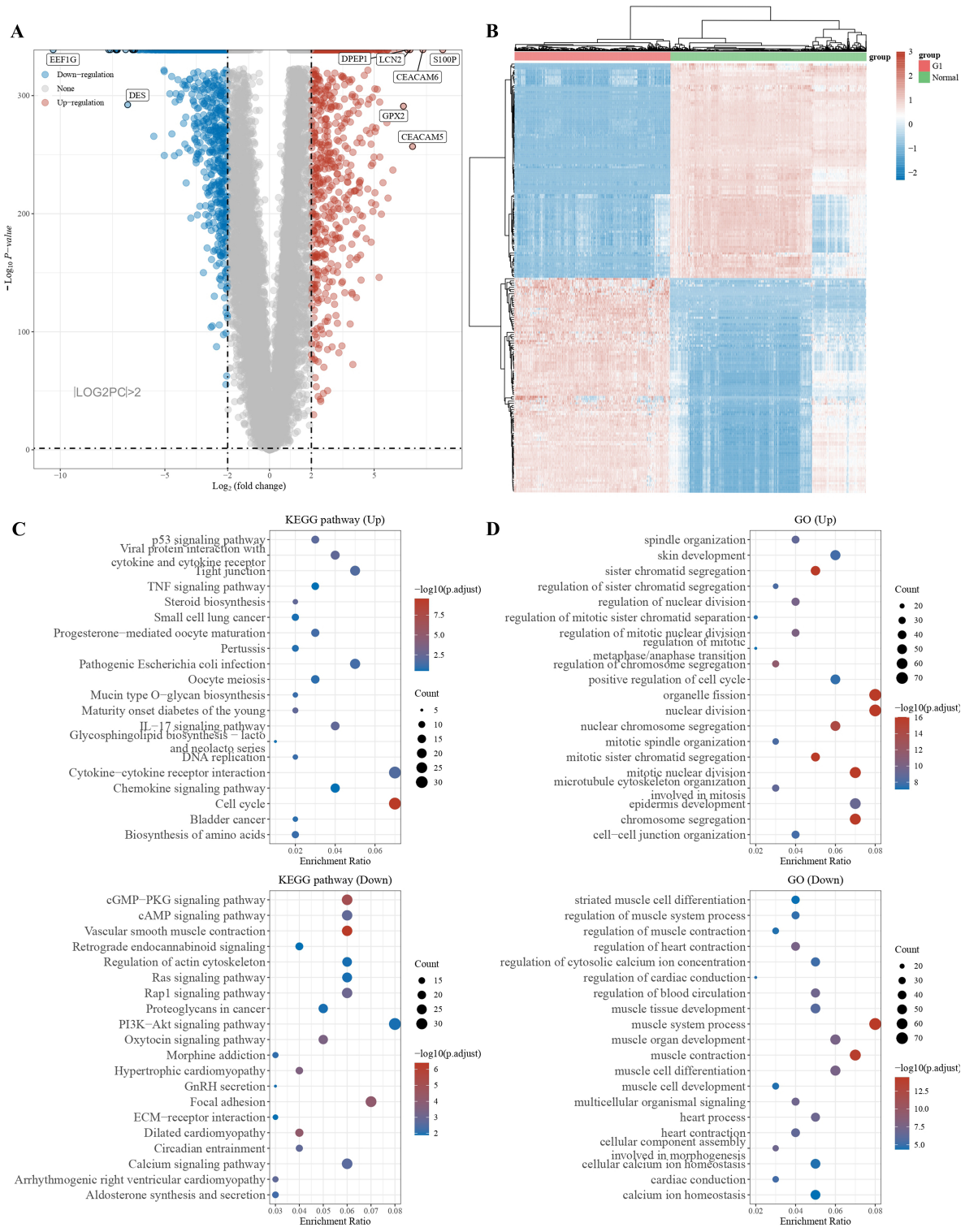

Fig. 1. Identification of DEGs between CRC patients and healthy controls. (A) Based on fold change and adjusted *p*-values, the volcano plot was developed, illustrating upregulated genes as red dots, downregulated genes as blue dots, and genes with insignificant ($p > 0.05$) as grey dots. (B) The heatmap represents the expression trends of genes in various tissues indicated by different colors. The figure shows the top 50 upregulated and the top 50 downregulated genes. (C) The enrichment analysis for differentially expressed genes revealed significant pathways within the KEGG database. (D) The GO term enrichment analysis for both upregulated and downregulated genes exhibited varying significance levels, as indicated by distinct colors. The circle's size indicates the number of genes involved, with bigger circles representing a higher gene count. DEGs, differentially expressed genes; CRC, colorectal cancer; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology.

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Table 1. The primers used in qRT-PCR.

Gene	Forward	Reverse
AGMAT	CTTGTCGAAGTTTCACCACCGTA	CTTTGGGGAGAGCACATAGCATC
GAPDH	CCCACTCCTCCACCTTTGAC	TCTTCCTCTTGTGCTCTTGC

AGMAT, agmatinase; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

Exclusion Criteria: The exclusion criteria for the study participants were as follows: (1) Patients with concurrent severe medical conditions such as severe cardiac, hepatic, or renal diseases, (2) those with a history of any other malignancies within the past five years, except those received adequate treatment for basal cell or squamous cell skin cancer or carcinoma in situ of the cervix, (3) the pregnant or breastfeeding women, (4) individuals experiencing psychiatric conditions or social circumstances, (5) and patients who have participated in any other clinical trial involving experimental treatment within the last 30 days.

Real-Time Quantitative Polymerase Chain Reaction

Total RNA from CRC tissues was extracted using TRIzol™ LS reagent (Cat#10-296-028, Invitrogen, Carlsbad, CA, USA) and subsequently converted into cDNA employing the SuperScript IV First-Strand Synthesis System (Cat#18090050, Invitrogen, Carlsbad, CA, USA). The *AGMAT* mRNA levels were determined through qRT-PCR using the PrimeScript RT kit (Cat#RR037B, Takara, Shiga, Japan) and PCR Master Mix (Cat#RR310B, Takara, Shiga, Japan). *AGMAT* relative expression levels were assessed using the $2^{-\Delta\Delta Ct}$ method. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was performed to normalize the RNA expression. The primers used in qRT-PCR are given in Table 1.

Statistical Analysis

The statistical analyses were performed using GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA). The data were expressed as the mean ± standard deviation (SD). A two-tailed Student's *t*-test was used to compare the two groups. However, multiple group comparisons were conducted employing a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for pairwise comparisons. Moreover, Pearson's correlation coefficient was used to evaluate the relationship among variables. A *p*-value of less than 0.05 was considered statistically significant. Additionally, the statistical test used for each analysis is indicated in the results section, alongside the corresponding findings.

Results

Identification of DEGs between CRC Patients and Healthy Controls

As shown in Fig. 1A, volcano plot analysis revealed 1917 differentially expressed genes (DEGs), including 928 upregulated and 989 downregulated genes. Moreover, the expression patterns of the 50 most significantly upregulated and 50 downregulated genes were visualized through the heatmap, indicating the most significant differential changes (Fig. 1B). The KEGG pathway enrichment analysis (Fig. 1C) revealed that DEGs were enriched in cell cycle and vascular smooth muscle contraction. Moreover, the GO analysis indicated their enrichment in organelle fission, nuclear division, and muscle tissue process (Fig. 1D).

Construction of a Prognostic Model for DEGs Signature

The top 200 DEGs were extracted and the correlation between the model score in high and low-risk groups and other factors such as immune score was assessed based on the construction of multiple gene signature prognostic models. Furthermore, tenfold cross-validated LASSO regression was conducted to identify additional genes significantly correlated with prognosis in CRC patients. The best λ value was obtained from the minimum partial likelihood deviation (λ_{min} = 0.025), associated with the 193 DEGs strongly associated with OS (Fig. 2A,B). Additionally, Cox regression analysis directly identified the best eight prognostic gene models (Matrix Metallopeptidase 1 (*MMP1*), Serine Protease Inhibitor Kazal-Type 1 (*SPINK1*), Cluster of Differentiation 24 (*CD24*), Serine Protease Inhibitor Kazal-Type 4 (*SPINK4*), *AGMAT*, EPH Receptor B2 (*EPHB2*), C2 Calcium-Dependent Domain Containing 4A (*C2CD4A*), Pituitary Tumor-Transforming Gene 1 (*PTTG1*), and proportional risk regression analysis was conducted to ascertain whether the prognosis of each gene held significant prognostic value for CRC patients. Furthermore, patients were categorized into high-risk and low-risk groups (Fig. 2C) and their survival status was determined collectively (Fig. 2D). The heat maps of the eight prognostic genes were developed (Fig. 2E).

Furthermore, Kaplan-Meier survival curves indicated that the high-risk group exhibited poorer overall survival (OS) than the low-risk group (Fig. 2F). Moreover, genetic prognostic features presented greater AUC in ROC analysis over time (Fig. 2G). These findings suggest that the multigene model enhances prediction accuracy for 1-year, 3-year, and 5-year overall survival rates.

AGMAT Expression was Increased in CRC Tissues

Analysis of TCGA gene expression data through GEPIA 2 revealed that AGMAT was upregulated in CRC tissues compared to the non-cancerous colon tissues (*p*

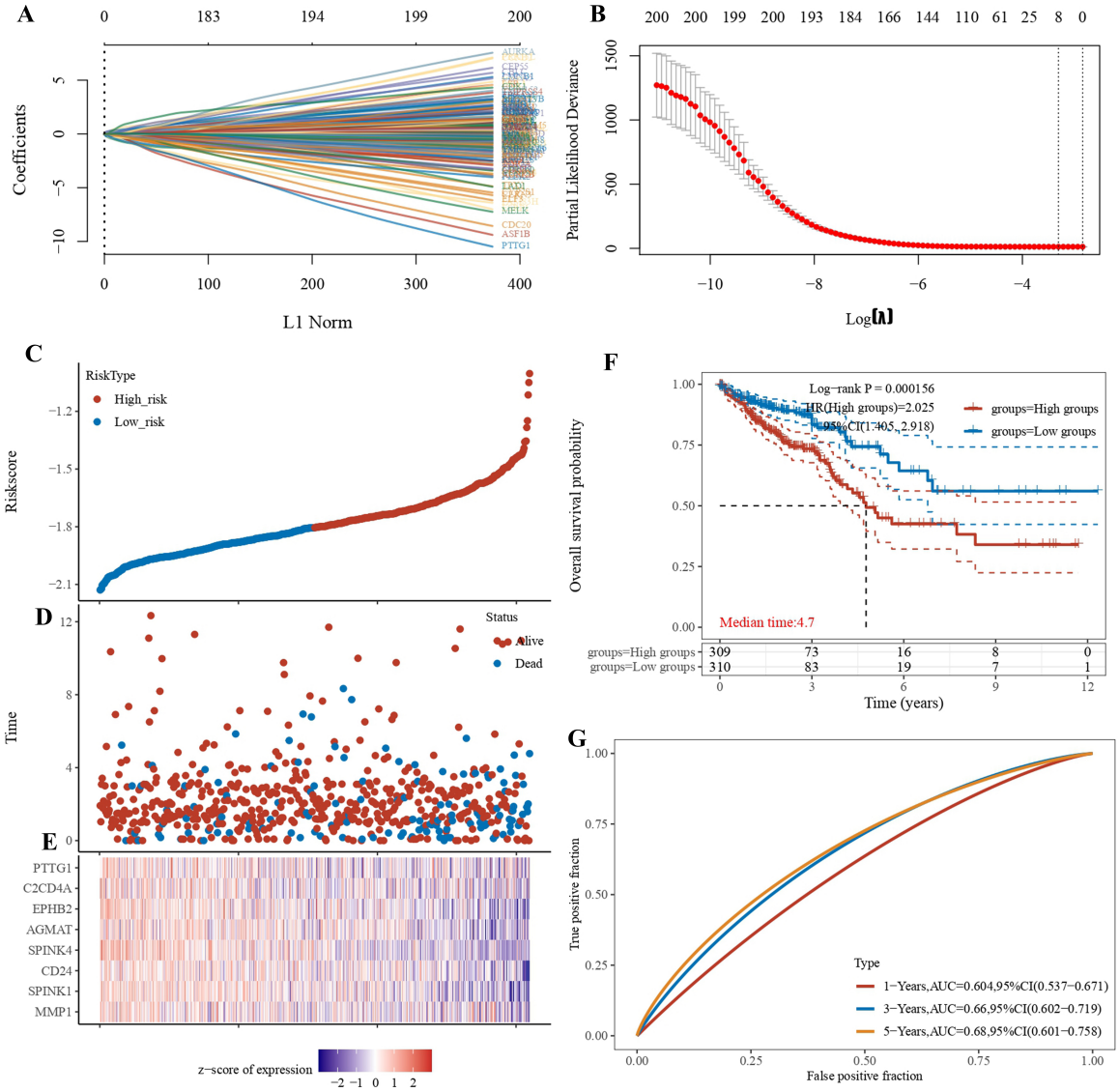

Fig. 2. Prognostic analysis of eight-gene signature in the training set. (A) The profiles of LASSO coefficients for 200 protein-coding genes were depicted. (B) Through LASSO regression applying tenfold cross-validation, 193 genes were identified as prognostic at the minimum lambda value. (C) A plot illustrated the distribution of risk scores. (D) The survival status of the patients. (E) A heatmap displayed the expression patterns of the eight prognostic genes across the low-risk and high-risk groups. (F) Kaplan-Meier survival curves analyzed the survival outcomes associated with the eight-gene signature. (G) The predictive accuracy of the eight-gene signature was evaluated using time-dependent receiver operating characteristic (ROC) analysis. LASSO, Least Absolute Shrinkage and Selection Operator.

< 0.05, Fig. 3A). Furthermore, the immunohistochemical analysis of HPA confirmed that the AGMAT expression was higher in CRC tissues (Fig. 3C) than in normal samples (Fig. 3B).

AGMAT Influenced the Prognosis of CRC

Subsequently, we explored the role of AGMAT on CRC prognosis using GEPIA 2. The prognostic analysis demonstrated that higher expression of AGMAT decreased OS ($p = 0.038$) and DFS ($p = 0.026$) in CRC patients (Fig. 4A,B).

AGMAT was Upregulated in Clinical CRC Samples

The tissues samples of CRC patients were collected to evaluate AGMAT expression. We observed that AGMAT expression was significantly elevated in CRC tissues (all $p < 0.05$, Fig. 5), aligning with the previous database prediction.

Discussion

Colorectal cancer (CRC) has posed a significant health challenge worldwide, ranking among the most fatal cancers [1]. Despite ongoing advances in treatment, the mortality

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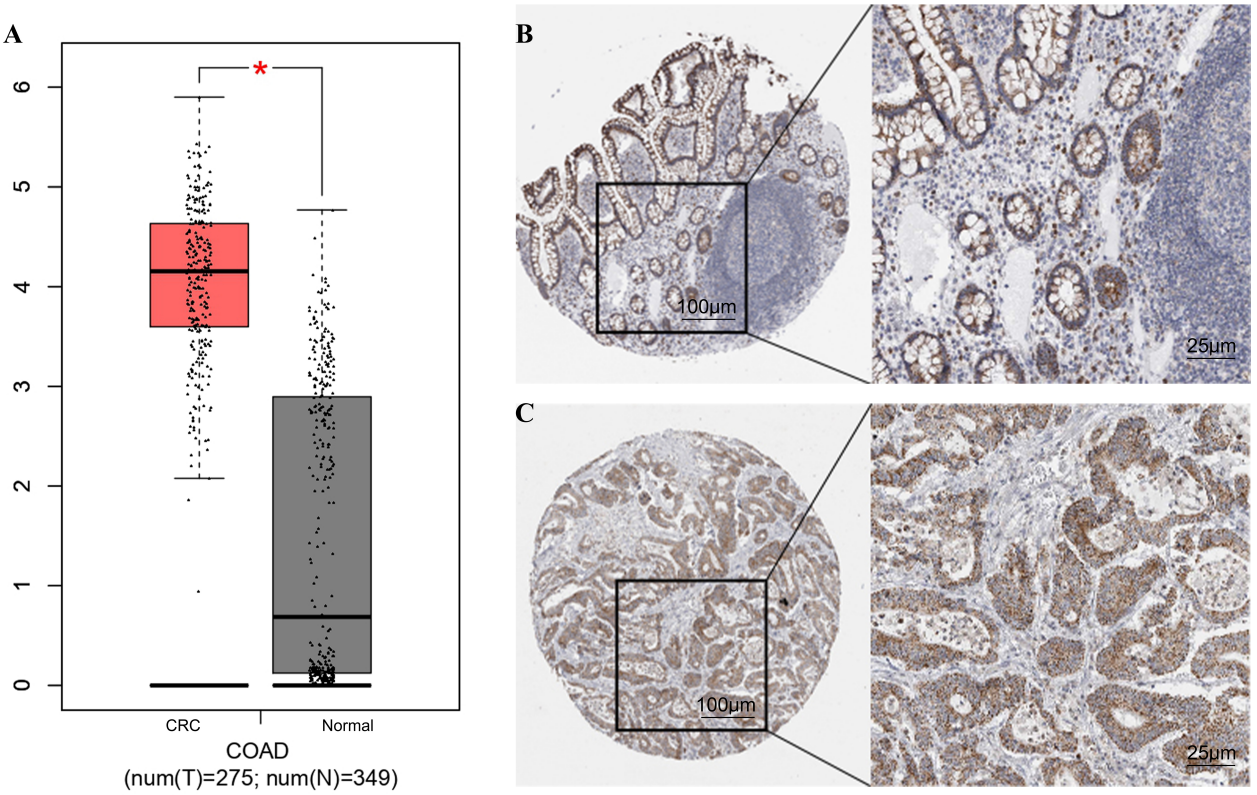

Fig. 3. AGMAT expression was increased in CRC tissues. (A) The GEPIA 2 database analysis of AGMAT expression levels in CRC compared to the normal group. *$p < 0.05$. Representative immunohistochemical staining images of (B) AGMAT in CRC and (C) normal tissues from the HPA database (Scale bar: = 100 μm, 25 μm). T, tumor; N, normal; HPA, Human Protein Atlas.

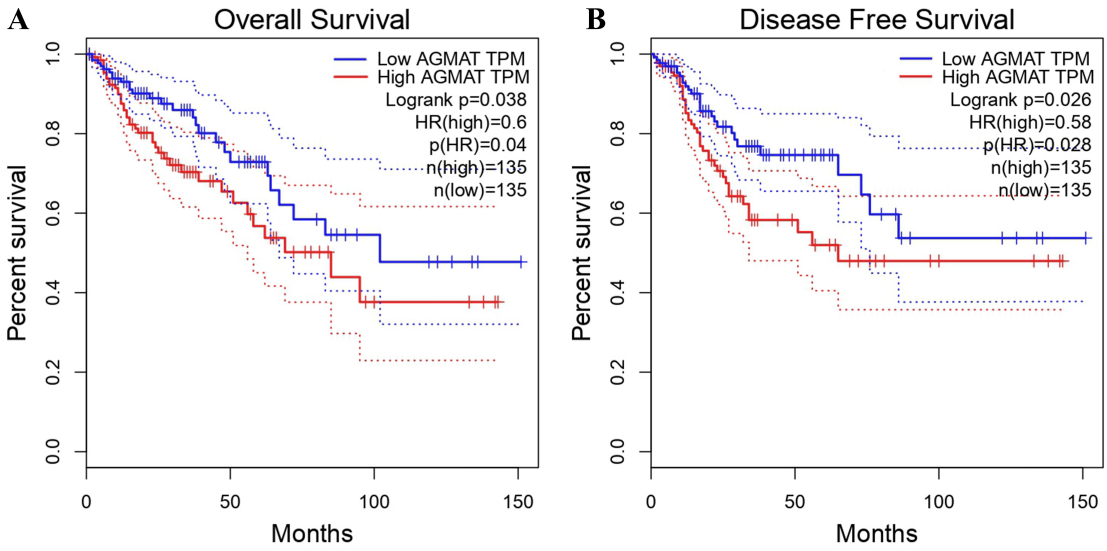

Fig. 4. Kaplan-Meier (KM) Plotter database and TCGA-based prognosis analysis. (A) OS analysis (KM plotter, TCGA data). (B) DFS analysis (KM plotter, TCGA data). TCGA, The Cancer Genome Atlas; OS, overall survival.

rate, particularly for metastatic colorectal cancer, remains high among cancer-related deaths. Inadequate early detection is among the possible factors. Therefore, it is imperative to identify new biomarkers for CRC diagnosis and prognosis.

In this study, we collected samples from TCGA and identified 928 upregulated and 989 downregulated differentially expressed genes (DEGs). Enrichment analysis using GO and KEGG pathways revealed that the upregulated DEGs were primarily associated with various processes,

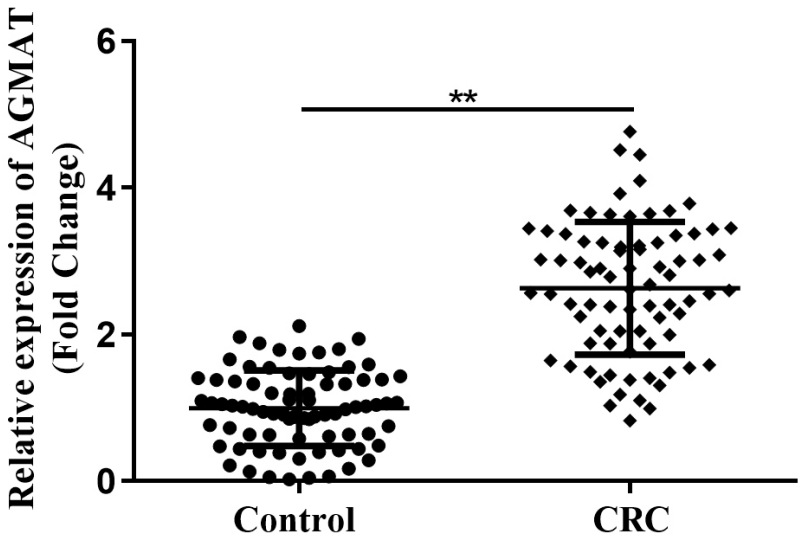

Fig. 5. AGMAT was upregulated in CRC tissues. The RNA expression of AGMAT in CRC tissues was detected using PCR. Note: $^{**}p < 0.01$.

including cell cycle, organelle fission, and nuclear division. The primary common downregulated DEGs were associated with the PI3K-AKT signaling pathway, vascular smooth muscle contraction, and muscle system process.

To identify genes associated with CRC prognosis, we proceeded with prognostic model analysis. Initially, the DEGs screened through the univariate Cox regression method in the training set were used in ten-fold cross-validated LASSO regression and BSR methods. Consequently, eight new DEGs (*MMP1*, *SPINK1*, *CD24*, *SPINK4*, *AGMAT*, *EPHB2*, *C2CD4A*, *PTTG1*) were screened. The order of the screening method and the content were referred to a previously outlined approach [22]. These eight novel genes were substantially associated with OS in CRC patients. After identifying eight prognostic genes, we developed eight genetic prognostic signatures and investigated their prognostic value in CRC patients. Our analysis indicated that patients categorized into the high-risk group exhibited a significantly poorer prognosis compared to those in the low-risk group.

Agmatinase (AGMAT) serves as a precursor of polyamine biosynthesis, exerting various biochemically significant effects on humans. Notably, AGMAT is known for its anti-convulsant, anti-inflammatory, and antidepressant properties, with essential neuromodulatory effects [23]. Additionally, AGMAT influences cell proliferation and viral replication in cancer [24]. AGMAT expression is upregulated in lung adenocarcinoma tissue, indicating its potential as a therapeutic marker for people with lung adenocarcinoma [25]. AGMAT expression is reduced in clear renal cell carcinoma [26]. Furthermore, increased expressions of arginine decarboxylase, ornithine decarboxylase, and agmatinase can serve as prognostic markers for detecting the occurrence of breast cancer [27]. Among studies related to CRC, only one has reported that changes in polyamine metabolism can modify the microenvironment of host tissues, resulting in chronic inflammation, impaired immunity, and elevated ROS levels, thereby affecting the progression of CRC [28].

We identified a potentially crucial gene and validated its expression and prognosis using the GEPIA database. The outcomes are consistent with the previous predictions. Finally, we performed additional expression validation utilizing the clinical samples we collected during this research.

Conclusions

This study revealed high AGMAT expression in CRC tissues. Importantly, high AGMAT levels correlated with poorer prognoses in CRC patients. The results indicate that AGMAT may serve as a potential biomarker for predicting survival rates in CRC, providing new insights into its contribution to the development of CRC and the possibilities for therapeutic interventions.

Availability of Data and Materials

All the data supporting the findings of this study have been included in the submission.

Author Contributions

DR, YX, BH and HL contributed to the study conception and design. DR did the material preparation. YX and BH did data collection and analysis. HL wrote and revised the manuscript and all authors made suggestions and revisons on the initial versions of the manuscript. The final

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manuscript was approved by all the authors. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study has obtained ethical approval from the Ethical Review Committee of Jincheng People's Hospital (Approval No: JC-2021-35). The study design adhered to the Declaration of Helsinki and patients submitted written informed consent before their inclusion in the study.

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Conflict of Interest

The authors declare no conflict of interest.

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