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Clinical implications and nomogram prediction of long noncoding RNA FRGCA as diagnostic and prognostic indicators in colon adenocarcinoma

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Abstract

Colorectal cancer, especially colon adenocarcinoma (COAD), is associated with significant morbidity and mortality worldwide. Long noncoding RNA (lncRNA) has been implicated in tumorigenesis. The aim of the present study was to elucidate the potential diagnostic and prognostic values of lncRNA FRGCA in COAD.

The data of 438 COAD patients were retrieved for analysis. Diagnostic significance was evaluated using tumor and nontumor tissues. Prognostic significance was evaluated using a Cox proportional regression model. Stratified analysis was performed to identify associations between clinical factors and lncRNA FRGCA expression. A nomogram was constructed using the clinical factors and lncRNA FRGCA for survival prediction. Enrichment analysis identified gene ontologies and metabolic pathways of mRNAs with high Pearson correlation coefficients with lncRNA FRGCA.

lncRNA FRGCA was highly expressed in tumor tissues of COAD and demonstrated diagnostic value (area under curve = 0.763, $P < .0001$). Prognostic significance analysis indicated that lncRNA FRGCA had prognostic value in COAD [adjusted $P < .001$, hazard ratio (HR) = 0.444, 95% confidence interval (95% CI) = 0.288–0.685] and high expression of lncRNA FRGCA indicated better survival in COAD. A nomogram was evaluated for prediction of survival at 1, 3, and 5 years. Enrichment analysis revealed many mRNAs involved in the structural constituents of the mitochondrial inner membrane and translational termination, protein binding, translation, ribosome, oxidative phosphorylation, and metabolic pathways, especially the nucleoplasm.

Differentially expressed in tumor vs nontumor tissues, lncRNA FRGCA had both diagnostic and prognostic implications in COAD, which may be associated with ribosome metabolism, oxidative phosphorylation, and nucleoplasm-related metabolic pathways.

Abbreviations: AUC = area under curve, BP = biological processes, CC = cellular components, CI = confidence interval, COAD = colon adenocarcinoma, CRC = colorectal cancer, DAVID = Database for Annotation, Visualization and Integrated Discovery, GGI = gene-gene interaction, HR = hazard ratio, KEGG = Kyoto Encyclopedia of Genes and Genomes, lncRNA = long noncoding RNA, MF = molecular functions, OS = overall survival, ROC = receiver operating characteristic.

Keywords: colon adenocarcinoma, FRGCA: long noncoding, prognosis, RNA: diagnosis

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The datasets analyzed during the current study are available from the corresponding author on reasonable request.

There are no patients were participated in the study except for patients in TCGA cohort. Thus, there is no need of ethics approval and consent to participate.

The authors report no conflicts of interest.

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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1. Introduction

Colorectal cancer (CRC) is the fourth most common tumor and the fourth most common cause of tumor-associated mortality worldwide, accounting for an estimated 1.4 million new cases and 700,000 deaths in 2012.[1,2] The continued development of modalities for the early diagnosis and prevention of CRC has tremendously benefited patient care. However, there were an estimated > 1.8 million new cases of CRC and 881,000 deaths in 2018, which accounted for about 10% of all newly diagnosed cancers and related deaths.[3] This tendency indicates the urgency for the early diagnosis and treatment of CRC. In addition, the 5-year relative survival rate of CRC patients remains at less than 50% in underdeveloped countries, whereas the survival rate is approximately 65% in developed countries, including Canada, the United States, Australia, and several European countries.[4–6] Surgical resection plays a significant role in the management of colon cancer.[7] For stage III colon adenocarcinoma (COAD), adjuvant systemic treatment is crucial to decrease the incidence of recurrence and increase the overall survival (OS) rate.[8,9] For patients with node-negative metastatic colon cancer, administration of adjuvant systemic treatment can greatly decrease tumor recurrence and increase patient survival.[10] However, the initiation of adjuvant systemic treatment is unnecessarily delayed in some cases.[11]

Long noncoding RNAs (lncRNAs), mRNA-like molecules that lack open reading frames, are highly evolutionarily conserved noncoding RNAs with lengths of > 200 nucleotides that have emerged as integral components of mammalian transcription.[12,13] Current studies have found that lncRNAs play crucial roles in the transcription of structural and functional proteins, thus the potential roles of lncRNAs have become an important focus in medical science.[13] Research suggests that lncRNAs play pivotal roles in a variety of cellular biological processes (BPs), especially in the regulation of cellular apoptosis,[14] proliferation, differentiation, and migration,[15] as well as genomic imprinting,[16] gene expression, posttranslational modification, and tumorigenesis.[17,18]

Many recent studies have investigated the clinical significance of lncRNAs in tumor progression. For instance, lncRNA SNHG7 is reportedly involved in varied pathological processes in different tumor types, such as hepatocellular carcinoma,[19] renal cell carcinoma,[20] and lung cancer,[21] in addition to CRC and COAD. Furthermore, lncRNA MALAT1 was found to promote cellular invasion and metastasis in CRC by sponging with microRNA-106b-5p[22] and lncRNA LINC01234 was reported to promote the expression of serine hydroxymethyltransferase 2 and cellular proliferation in colon cancer.[23] However, the roles of lncRNA FRGCA in tumorigenesis remain relatively unknown. Therefore, the aim of the present study was to explore the potential utility of lncRNA FRGCA as a diagnostic and prognostic indicator of COAD.

2. Material and methods

2.1. Patient data

The cohort of this retrospective study was limited to patients with pathologically confirmed COAD. Data regarding the expression profiles of lncRNA FRGCA were download from The Cancer Genome Atlas database (https://cancergenome.nih.gov/) and were normalized using the DESeq module of the R/Bioconductor package.[24] Clinical data coupled to the expression data of mRNAs, miRNAs, and lncRNAs related to COAD were downloaded from the Oncolnc database (http://www.oncolnc.org/).

2.2. Analysis of diagnostic and prognostic significance

To determine the diagnostic significance of lncRNA FRGCA in COAD, the expression profiles of tumor and nontumor tissues were determined, and a receiver operating characteristic (ROC) curve was constructed. An area under curve (AUC) of ≥0.7 was considered to demonstrate diagnostic significance. On the basis of the expression patterns of lncRNA FRGCA in tumor tissues, the COAD patients were divided into 2 groups: low vs high lncRNA FRGCA expression. Kaplan–Meier plots were constructed to reveal the prognostic significance of lncRNA FRGCA in COAD. Adjusted probability (P) values were calculated to determine whether lncRNA FRGCA was significant for the prognosis of COAD. Clinical factors with P values $< .5$ were considered significant and thus used for adjusted survival analysis.

2.3. lncRNA FRGCA expression during COAD progression and forest plot construction

Cases were divided into 4 groups according to tumor stage (I–IV) and t tests were performed to identify differences in lncRNA FRGCA expression levels between stage I vs II, III, and IV. Here, significant P values demonstrated that lncRNA FRGCA expression was associated with COAD progression. A forest plot was constructed using GraphPad software, version 7.0 (GraphPad Software, Inc., La Jolla, CA), to visualize hazard ratios (HRs) and P values of each factor associated with COAD progression.

2.4. Stratified analysis and nomogram construction of clinical factors and lncRNA FRGCA

Stratified analysis of clinical factors was performed to determine the prognostic significance of lncRNA FRGCA in COAD. The following factors were included for analysis: sex (male vs female); age (≤60 vs >60 years); and tumor stage (I vs II, III, and IV).

Nomograms were constructed based on age, sex, tumor stage, and lncRNA FRGCA expression to predict the 1-, 3-, and 5-year OS rates of COAD patients. Each factor was assigned a score, which were summed for survival prediction.

2.5. Identification of Pearson correlated mRNAs, enrichment analysis, and construction of gene-gene interaction (GGI) network models of relevant mRNAs

The Pearson correlation coefficient (r) was evaluated to identify correlations between lncRNA FRGCA and mRNAs throughout the genome using R version 3.5.0 (https://www.r-project.org/). The mRNAs correlated with lncRNA FRGCA ($r \geq .25$) were used for further analysis. Enrichment analysis of these mRNAs was performed to identify Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and gene ontology, especially those related to the functions of BPs, cellular components (CCs), and molecular functions (MFs), using the online Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/home.jsp) version 6.8.[25,26] A GGI network was constructed to visualize the interactions among the mRNAs of interest using the GeneMANIA plugin of the

Cytoscape software platform,[27,28] while visualized BP, CC, and MF analyses were performed using the BinGO plugin.[29]

2.6. Statistical analysis

Statistical analysis was performed using SPSS Statistics for Windows, version 17.0 (SPSS, Inc., Chicago, IL). The Kaplan–Meier method was used to determine the median survival time, while the log-rank test was used to provide *P* values and identify differences among groups. Both univariate and multivariate Cox proportional hazards regression models were constructed, and 95% confidence intervals (95% CIs) and HRs were calculated. A *P* value $\leq$.05 was considered statistically significant.

3. Results

3.1. Demographic characteristic of COAD patients

The study cohort included a total of 438 COAD patients (234 males and 204 females), of whom 134 were aged $\leq$60 years and 302 were aged >60 years (age data were missing for two patients). In regard to tumor stage: 73 were classified as stage I, 167 as stage II, 126 as stage III, and 61 as stage IV (tumor stage data was missing for 3 patients). Among these factors, only tumor stage was significantly correlated to OS (Table 1). Thus, the multivariate Cox proportional hazards regression model was further adjusted for tumor stage.

3.2. The diagnostic and prognostic significance of lncRNA FRGCA expression in regard to disease progression

As compared to nontumor tissues, lncRNA FRGCA was highly expressed in tumor tissues (Fig. 1A, $P<.001$) and was differentially expressed in the low vs high group (Fig. 1B, $P<.001$).

In the diagnostic significance analysis, a ROC curve showed that the AUC of lncRNA FRGCA was 0.763 with a sensitivity (Se) of 97.56% and specificity (Sp) of 52.08%, demonstrating diagnostic significance of lncRNA FRGCA in COAD (Fig. 2A, $P<.0001$, 95% CI=0.711–0.815). Although the univariate Cox proportional hazards regression model showed that lncRNA FRGCA had no prognostic value in COAD (crude $P=.086$), the multivariate model did reveal prognostic value (adjusted $P<.001$, HR=0.444, 95% CI=0.288–0.685, Table 2, Fig. 2B). These results showed that high expression of lncRNA FRGCA indicated better survival in COAD.

3.3. Stratified analysis of clinical factors

Stratified analysis was performed to further explore the prognostic significance of these factors in relation to lncRNA FRGCA in COAD. Kaplan–Meier plots of sex (male vs female), age ($\leq$60 vs >60 years), and tumor stage (I vs II, III, and IV) are presented in Figure 3 and Table 3. The factors male, female, age $\leq$60 and >60 years, and tumor stages II and IV were associated

Table 1

Demographic characteristics of COAD patients.

variables	Patients (n=438)	No. of events	MST (days)	HR (95% CI)	Log-rank *P*
Gender					
Male	234	54	2475	Ref.	.545
Female	204	44	NA	0.884 (0.593–1.318)	
Age*					
$\leq$60	134	24	NA	Ref.	.255
>60	302	73	2475	1.307 (0.824–2.075)	
Tumor stage†					
I	73	4	NA	Ref.	**<.0001**
ii	167	27	2821	2.240 (0.781–6.421)	.133
iii	126	31	NA	**4.068 (1.434–11.538)**	**.008**
iv	61	31	858	**11.291 (3.980–32.026)**	**<.0001**

95% CI=95% confidence interval, COAD=colon adenocarcinoma, HR=hazard ratio, MST=median survival time.

* Two patients data were missing in age.

† Three patients data were missing in tumor stage.

Bold indicates significant *P* value.

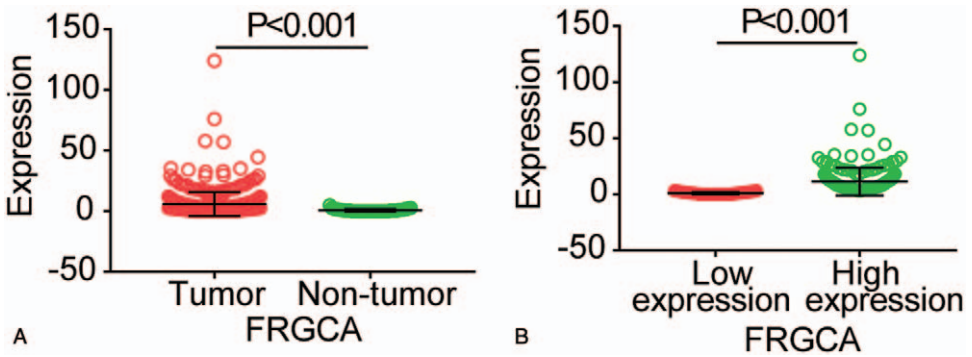

Figure 1. Scatter plots and Pearson correlation plot. (A) Scatter plot of lncRNA FRGCA in tumor and nontumor tissues; (B) Scatter plot of lncRNA FRGCA in low and high expression groups.

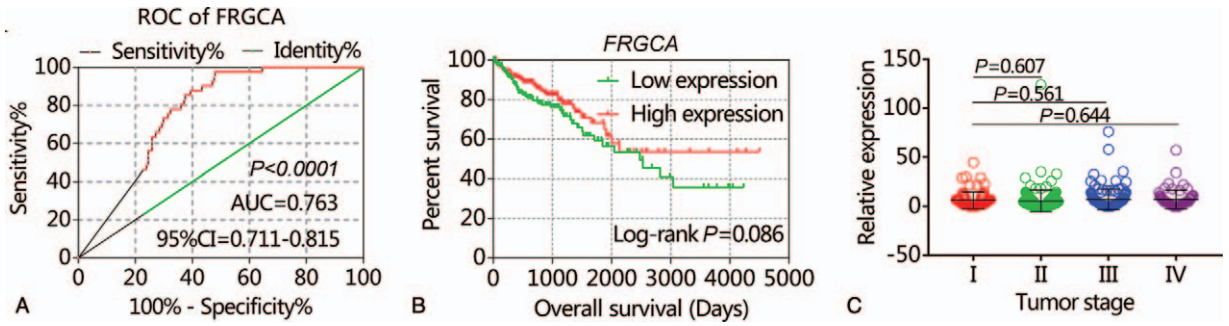

Figure 2. Diagnostic receiver operating curve, Kaplan–Meier plot, scatter plot, and coexpression plot. (A) Diagnostic receiver operating curve of lncRNA FRGCA; (B) Kaplan–Meier plot of lncRNA FRGCA; (C) Scatter plot of lncRNA FRGCA expression with tumor stage; (D) Coexpression network of lncRNA FRGCA-related mRNAs.

with the OS of COAD patients (all, adjusted $P < .05$). In addition, a forest plot was constructed to visualize the HR and *P* values of these factors (Fig. 4).

3.4. Nomogram for prediction of COAD prognosis

Nomograms were constructed using the factors age, sex, tumor stage, and lncRNA FRGCA expression. As shown in Figure 5, the factors female, age > 60 years, tumor stages III and IV, and low expression of lncRNA FRGCA had higher scores than male, age ≤60 years, tumor stages I and II, and high expression of lncRNA FRGCA. Differences in total scores indicated variations in the prediction of 1-, 3-, and 5-year OS, where a higher score was correlated to poorer prognosis at all time points. Moreover, these scores indicated that OS was best at 1 year and poorest at 5 years.

3.5. Identification of Pearson correlated mRNAs, enrichment analysis, and GGI network of mRNAs

Pearson correlation was calculated between lncRNA FRGCA and PCGs. Detailed mRNA names and coefficients of correlation are listed in Supplementary Table 1, http://links.lww.com/MD/F43. A GGI network of coexpression relationships among these mRNAs is shown in Figure 2D.

Enrichment analysis using DAVID revealed that the BP, CC, and MF functions were enriched in regard to the mitochondrial inner membrane, mitochondrion, mitochondrial translational elongation, mitochondrial translational termination, structural constituents of the ribosome, protein binding, translation, ribosome, and nucleoplasm, among others (Fig. 6A, B, D). The following KEGG pathways were enriched: ribosome, Huntington disease, metabolic pathways, pyrimidine metabolism, oxidative phosphorylation, Alzheimer disease, Parkinson disease, RNA polymerase, nonalcoholic fatty liver disease, and the sulfur relay system, among others (Fig. 6C). Detailed enrichment results of BP, CC, and MF, and KEGG pathways are shown in Supplementary Tables 2, http://links.lww.com/MD/F44 and 3, http://links.lww.com/MD/F45, respectively.

Furthermore, the visualized BP, CC, and MF results are shown in Figures 7–9, respectively. The BP-related functions included cellular metabolic processes, primary metabolic processes, CC organization, oxidative phosphorylation, ribonucleoprotein complex biogenesis, protein complex assembly, and RNA modification, among others (Fig. 7). The CC-related functions included intracellular membrane-bound organelles, cytoplasm, large ribosomal subunit, mitochondrion, intracellular part, intracellular organelle, mitochondrial envelope, and cytoplasmic part and organelle lumen, among others (Fig. 8). The MF-related functions included structural constituents of the ribosome, structural molecule activity, nuclease activity, RNA polymerase activity, hydrolase activity, hydrolyzing N-glycosyl compounds, NADH dehydrogenase activity, oxidoreductase activity, acting on NADH or NADPH, and quinone or similar compounds as the acceptor, among others (Fig. 9).

4. Discussion

The results of the present study revealed that lncRNA FRGCA was differentially expressed in COAD tumor and nontumor tissues. Further analysis demonstrated that lncRNA FRGCA had diagnostic and prognostic significance in COAD. High expression of lncRNA FRGCA indicated better survival in COAD patients. In addition, stratified analysis found that sex, age, and tumor stage were associated with lncRNA FRGCA expression. Moreover, a nomogram was constructed using sex, age, tumor stage, and lncRNA FRGCA expression to predict 1-, 3-, and 5-year OS rates. However, tumor stages II, III, and IV were not associated with lncRNA FRGCA expression.

Table 2

Survival analysis of CRC patient by *FRGCA*.

LncRNA expression	Patients/events	MST, d	Crude HR (95% CI)	Crude *P*	Adjusted HR (95% CI)	Adjusted *P*[*]
FRGCA						
Low	219/58	2475	Ref.	.086	Ref.	**<.001**
High	219/40	NA	0.703 (0.470–1.052)		**0.444 (0.288–0.685)**	

95% CI = 95% confidence interval, HR = hazard ratio, MST = median survival time, Ref. = reference.
[*] *P* was adjusted for stage.
Bold indicates significant *P* value.

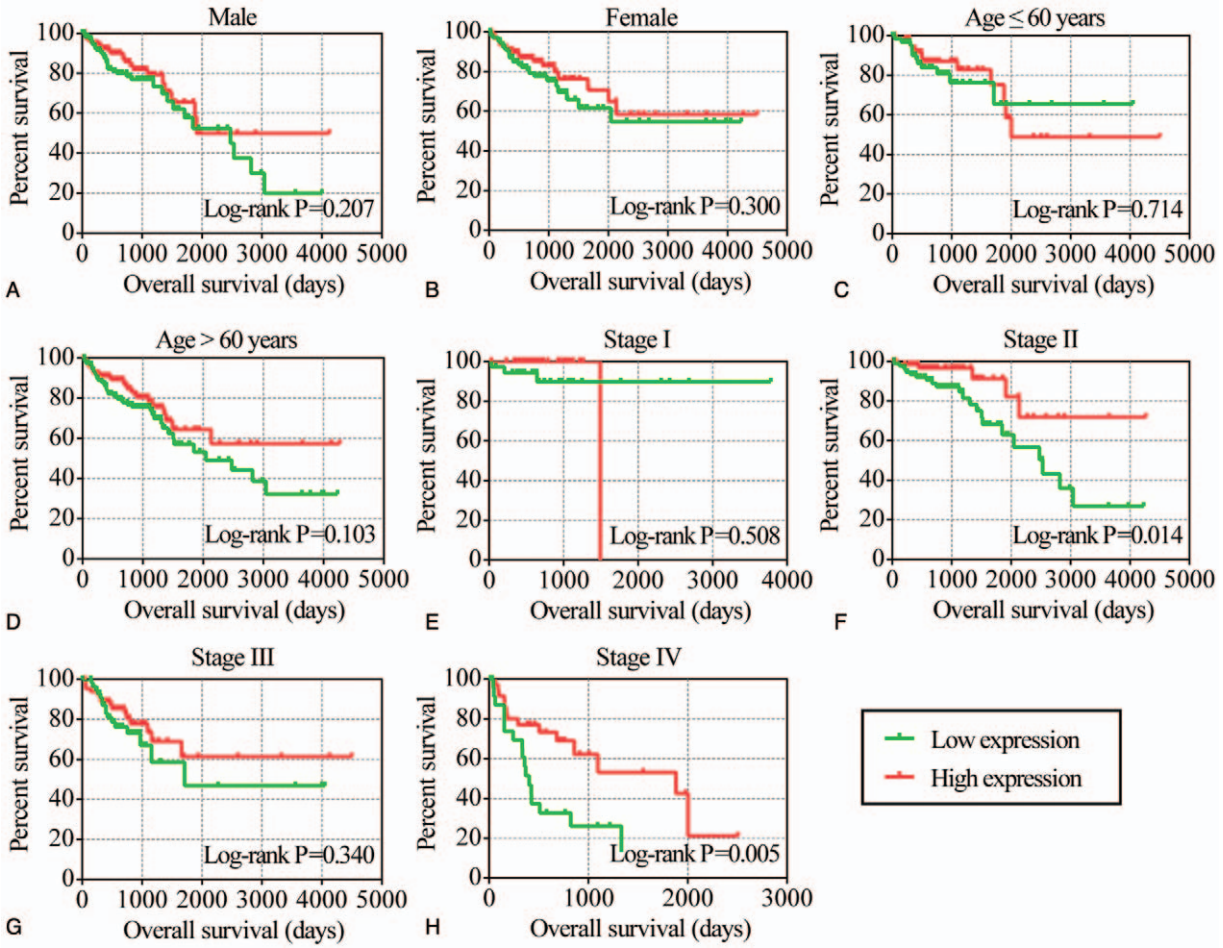

Figure 3. Kaplan–Meier plots of sex, age, and tumor stage. (A) Kaplan–Meier plot of male; (B) Kaplan–Meier plot of female; (C) Kaplan–Meier plot of age ≤60 yrs; (D) Kaplan–Meier plot of age > 60 yrs; (E) Kaplan–Meier plot of tumor stage I; (F) Kaplan–Meier plot of tumor stage II; (G) Kaplan–Meier plot of tumor stage III; (H) Kaplan–Meier plot of tumor stage IV.

Pearson correlation was employed to identify correlations with mRNAs throughout the genome, which were than selected for enrichment analysis. Enrichment analysis of BP, CC, and MF indicated that these mRNAs were involved in mitochondrial inner membrane and translational termination, structural constituents of the ribosome, protein binding, translation, ribosome, and nucleoplasm, among others. Enrichment analysis of KEGG pathways indicated that these mRNAs were involved in

Table 3

Stratified analysis of clinical factors with *FRGCA*.

	FRGCA			
Variables	**Low**	**High**	**Adjusted HR (95% CI)**	**Adjusted *P***
Gender				
Male	112/32	122/22	**0.489 (0.272–0.878)**	**.017**
Female	107/26	97/18	**0.388 (0.200–0.751)**	**.005**
Age*				
≤60	68/12	66/12	**0.303 (0.110–0.831)**	**.020**
>60	150/45	152/28	**0.513 (0.312–0.841)**	**.008**
Tumor stage†				
I	37/3	36/1	0.465 (0.046–4.740)	.518
ii	94/22	73/5	**0.316 (0.119–0.835)**	**.020**
iii	58/16	68/15	0.710 (0.349–1.441)	.342
iv	25/17	36/14	**0.365 (0.174–0.768)**	**.008**

95% CI = 95% confidence interval; HR = hazard ratio.
* Two patients data were missing in age.
† Three patients data were missing in tumor stage.
Bold indicates significant *P* value.

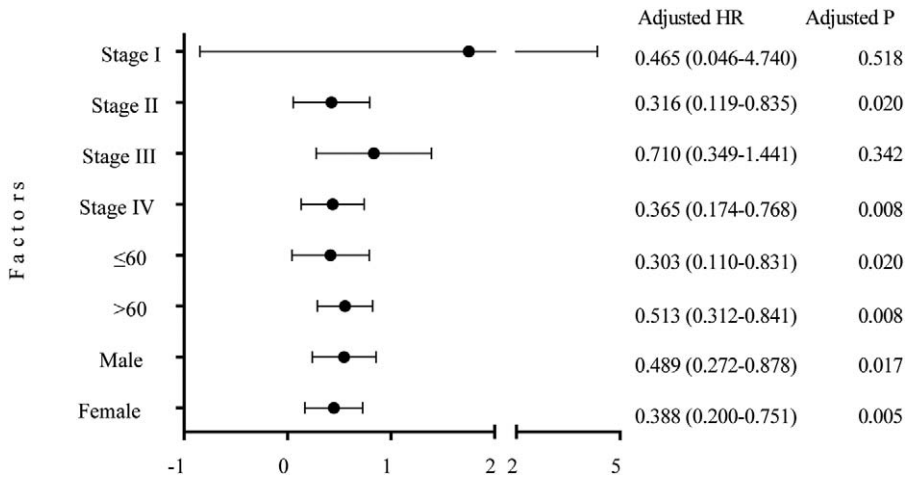

Figure 4. Forest plot of age, sex, tumor stage, and lncRNA FRGCA expression-related prognosis.

ribosome, oxidative phosphorylation, metabolic pathways, pyrimidine metabolism, RNA polymerase, nonalcoholic fatty liver disease, and the sulfur relay system, among others. Therefore, we hypothesized that lncRNA FRGCA was associated with the diagnosis and prognosis of COAD via oxidative phosphorylation, ribosome metabolism, and metabolic pathways, especially in the nucleoplasm.

In 2002, Okazaki et al[30] first reported the involvement of lncRNAs in mammalian transcription. Since then, the importance of these nonprotein coding molecules has been widely investigated.[30] For example, in the Encyclopedia of DNA Element study, Djebali et al[31] revealed that the human genome has more than 9640 lncRNA loci, which accounted for approximately one-half of all protein-coding genes. The findings of prior studies have gradually altered our attitude and understanding of the mammalian genome and raised new questions of the potential functions of lncRNAs.[13] The biogenesis of lncRNAs parallels that of mRNAs, as a vast proportion are transcribed by RNA polymerase II and have a 5' cap and 3' polyadenylation signal.[13] Combined with prior analysis of transcriptional outputs, sequencing of the human and mouse genomes indicated that approximately 80% of the transcription of the mammalian genome occurs in a cell-specific manner, which gave rise to a new understanding of transcriptional modulation, especially within noncoding regions.[31,32]

The use of integrated methodologies with the continued progress in RNA sequencing methods has facilitated the discovery and identification of many new lncRNAs. However, other than carcinoembryonic antigen and carbohydrate antigen 19–9, there is currently a lack of biomarkers for the early diagnosis of CRC, primarily because of the relatively low Sp and Se.[33–36] A previous molecular study of stool samples has identified several diagnostic biomarkers with relatively high Se and Sp for colon cancer.[37] The many advantages of scatological

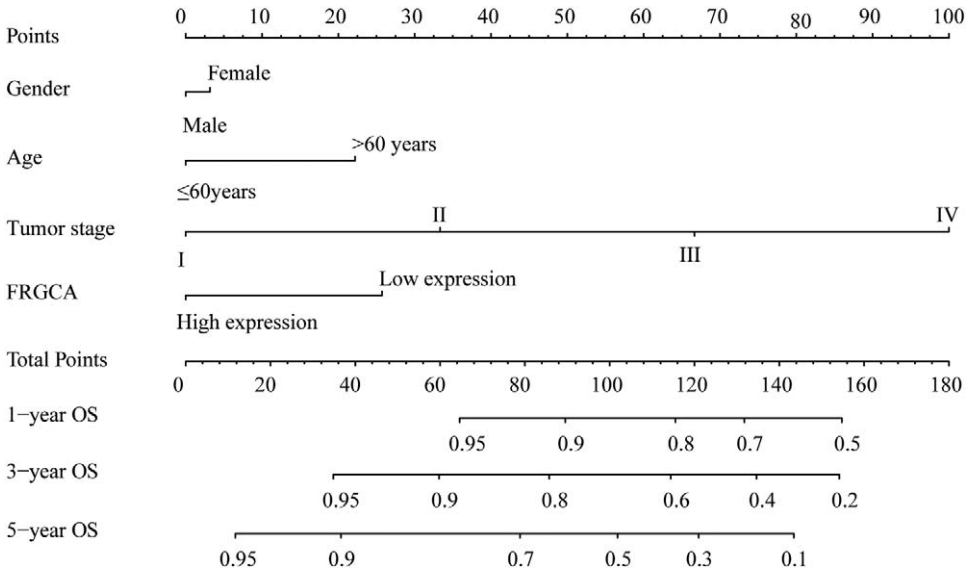

Figure 5. Nomogram constructed using lncRNA FRGCA expression, age, sex, and tumor stage for prediction of survival of COAD patients.

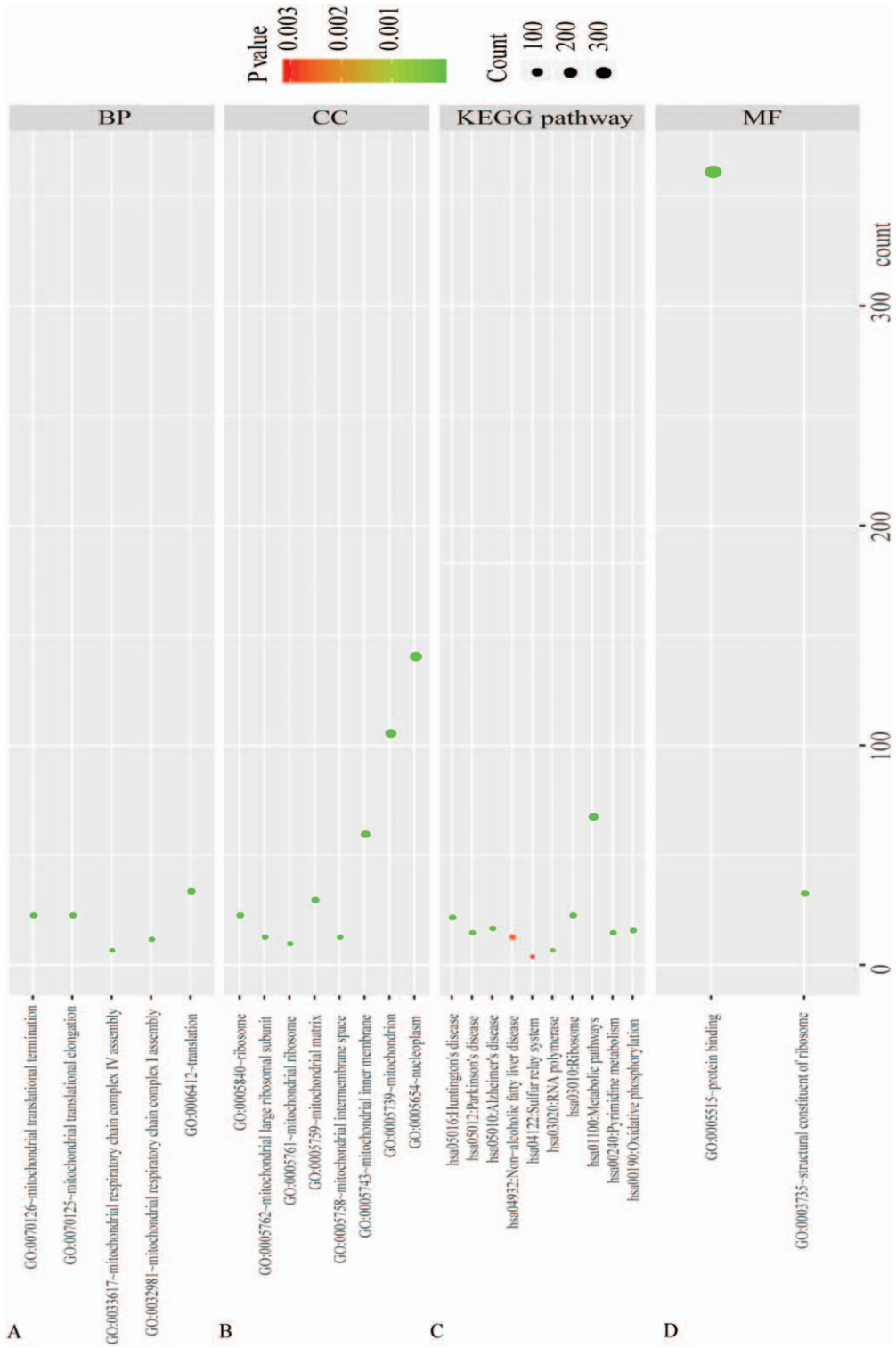

Figure 6. Enrichment results of gene ontologies and metabolic pathways. (A) Enrichment results of biological processes; (B) Enrichment results of cellular components; (C) Enrichment results of metabolic pathways; (D) Enrichment results of molecular functions.

studies include no requisite of bowel preparation, testing on an outpatient basis, the noninvasive nature of the assays, ease of performance, and no risk of complications that commonly occur with colonoscopy, especially perforation and bleeding. Hence, the use of fecal samples for colon cancer screening offers a noninvasive and relatively inexpensive option to the patient.[37] More surprisingly, these fecal immunochemical tests have greater Se and Sp than colonoscopy. In fact, Weller et al[38] reported a Se of 83% and Sp of 96% for screening of CRC among 6208 subjects; Rozen et al[39] reported a Se of 86% and Sp of 98% for

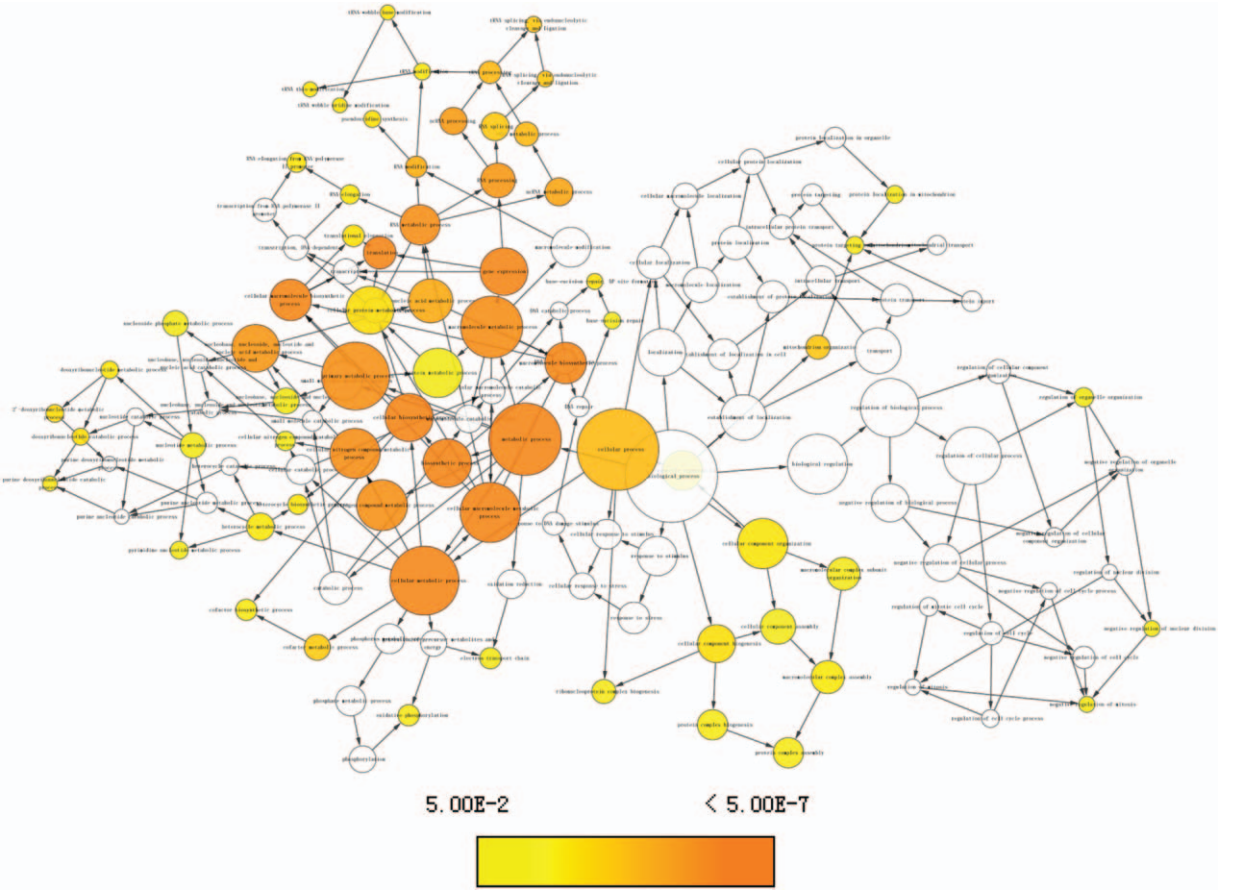

Figure 7. Visualized biological process results using lncRNA FRGCA-related mRNAs.

screening of CRC and advanced adenoma (AA) in 403 subjects; Wong et al[40] reported a Se of 62% and Sp of 93% for screening of CRC and AA among 250 subjects at a cutoff of 70 standard units; Lohsiriwat et al[41] reported a Se of 91% and Sp of 94% for screening of CRC among 164 subjects; and Rozen et al[42] reported a Se of 69% and Sp of 92% for screening of AA or CRC among 330 subjects at a cutoff value of 50 ng/dL. Meanwhile, the results of the present study showed that lncRNA FRGCA had diagnostic significance with AUC of 0.763, with a Se of 97.56% and Sp of 52.08% among 438 COAD patients. Although there were differences from obvious COAD tissues, our results lead to a novel finding of lncRNA FRGCA as a potential diagnostic biomarker for COAD. Of course, the diagnostic significance of lncRNA FRGCA must be further validated in multicenter studies.

Colon cancer-associated transcript-1 (CCAT1), an lncRNA containing 2628 bp, is located on chromosome 8q24.21 and has been reported to facilitate the progression of colon cancer, as it was abnormally expressed in tumor tissues.[43] Another investigation revealed that CCAT1 transcription was stimulated by c-Myc, and then facilitated the growth and invasion capacities of colon cancer cells.[44] CCAT1 expression was notably increased in the early and late stage of colon cancer with significant upregulated expression in adenomatous polyps, the tumor-proximal colonic epithelium, sites of liver metastasis, and the lymph nodes.[45] In addition to prognosis, CCAT1 expression is reportedly associated with tumor stage, local infiltration depth, vascular invasion, and carbohydrate antigen 19–9 levels.[46]

Furthermore, the functional characteristics of CCAT1 in other tumors have also been explored. Previous studies have reported that CCAT1 expression is consistently upregulated and functions as an oncogene in cancers of the stomach,[47,48] liver,[49,50] gallbladder,[51] ovary,[52] breast,[53] and lung.[54,55] Moreover, CCAT1 was overexpressed in hepatocellular carcinoma tissues and promoted cell invasion, proliferation, and migration in vitro.[56] CCAT1 has been shown to promote cell proliferation in lung cancer.[54] Once activated by c-Myc, CCAT1 promotes the progression of gastric cancer,[47] while overexpression of CCAT1 in breast cancer tissues has been associated with tumor-node-metastasis staging, lymph node metastasis, and tumor differentiation, as indicated by poor OS and tumor progression-free survival.[45] To the best of our knowledge, this is the first reported association between lncRNA FRGCA expression and tumor progression. Our results confirmed the prognostic significance of lncRNA FRGCA in COAD, as high expression was correlated with better OS. However, in this study, there was no association between lncRNA FRGCA expression and tumor stage. As with other lncRNAs, CCAT1 has been associated with various other tumors, thus we speculate that lncRNA FRGCA expression could be used as a prognostic indicator with other tumors as well, although further studies are needed to explore such possibilities.

Abnormal regulation of lncRNAs has been shown to contribute to tumor pathologies, which also provide a new clue for the lncRNA-based treatment.[57–59] The results of the present study revealed that lncRNA FRGCA-related mRNAs were

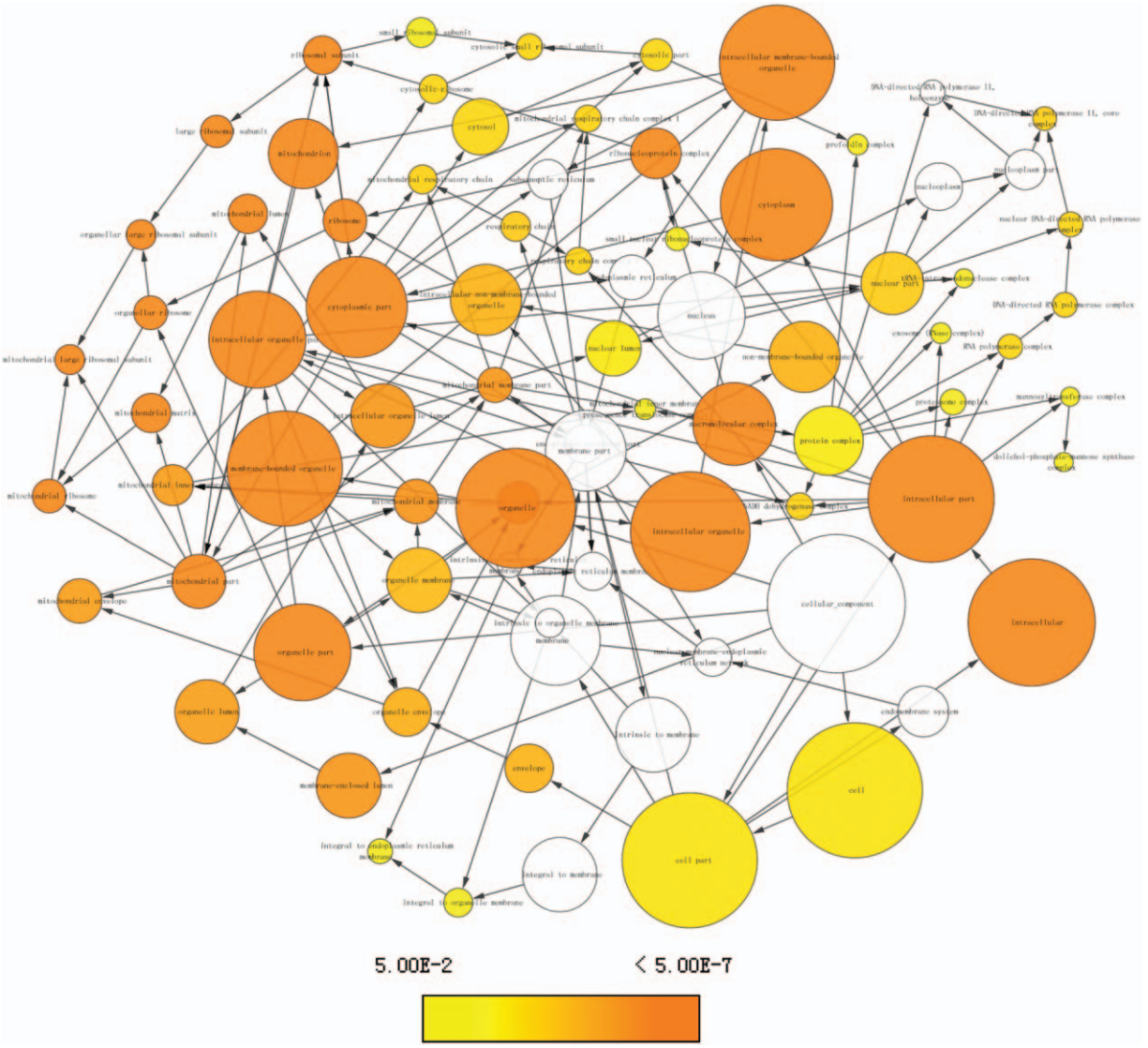

Figure 8. Visualized cellular component results using lncRNA FRGCA-related mRNAs.

enriched in the processes of mitochondrial inner membrane and translational termination, structural constituents of the ribosome, protein binding, translation, neoplasm, oxidative phosphorylation, metabolic pathways, and RNA polymerase, among others. Therefore, we speculate that lncRNA FRGCA expression is a suitable indicator for the diagnosis and prognosis of COAD, based on the relationships with oxidative phosphorylation, ribosome metabolism, metabolic pathways, especially in the nucleoplasm, although further functional trials are needed for validation of these findings.

There were some limitations to this study that should be addressed. First, the study cohort was relatively limited, thus a larger population is needed to validate the diagnostic and prognostic significance of lncRNA FRCGA in COAD. Second, the clinical value of lncRNA FRCGA on COAD must be investigated in multicenter studies across countries and regions. Third, further functional trials are needed to explore the specific mechanisms underlying the involvement of lncRNA FRCGA in COAD and to investigate potential associations between lncRNA FRGCA and the progression of other tumors. In addition, consensus molecular subtypes should be performed for further understanding the mechanism of COAD in future studies.

5. Conclusion

The results of the present study reported, for the first time, associations between lncRNA FRGCA expression and COAD prognosis and diagnosis. In addition, potential mechanisms were identified. We found that lncRNA FRGCA expression was greater in tumor tissues than nontumor tissues, suggesting diagnostic and prognostic value. Furthermore, a nomogram was created to predict the 1-, 3-, and 5-year OS rates. Functional enrichment analysis of genome-wide lncRNA FRGCA-related mRNAs revealed involvement in mitochondrial translational termination, ribosome, protein binding, translation, oxidative phosphorylation, metabolic pathways, among others. Therefore, we speculate that the associations between lncRNA FRGCA and COAD occur through ribosome metabolism, oxidative phosphorylation, and nucleoplasm-related metabolic pathways. Nonetheless, further studies are needed to validate these results

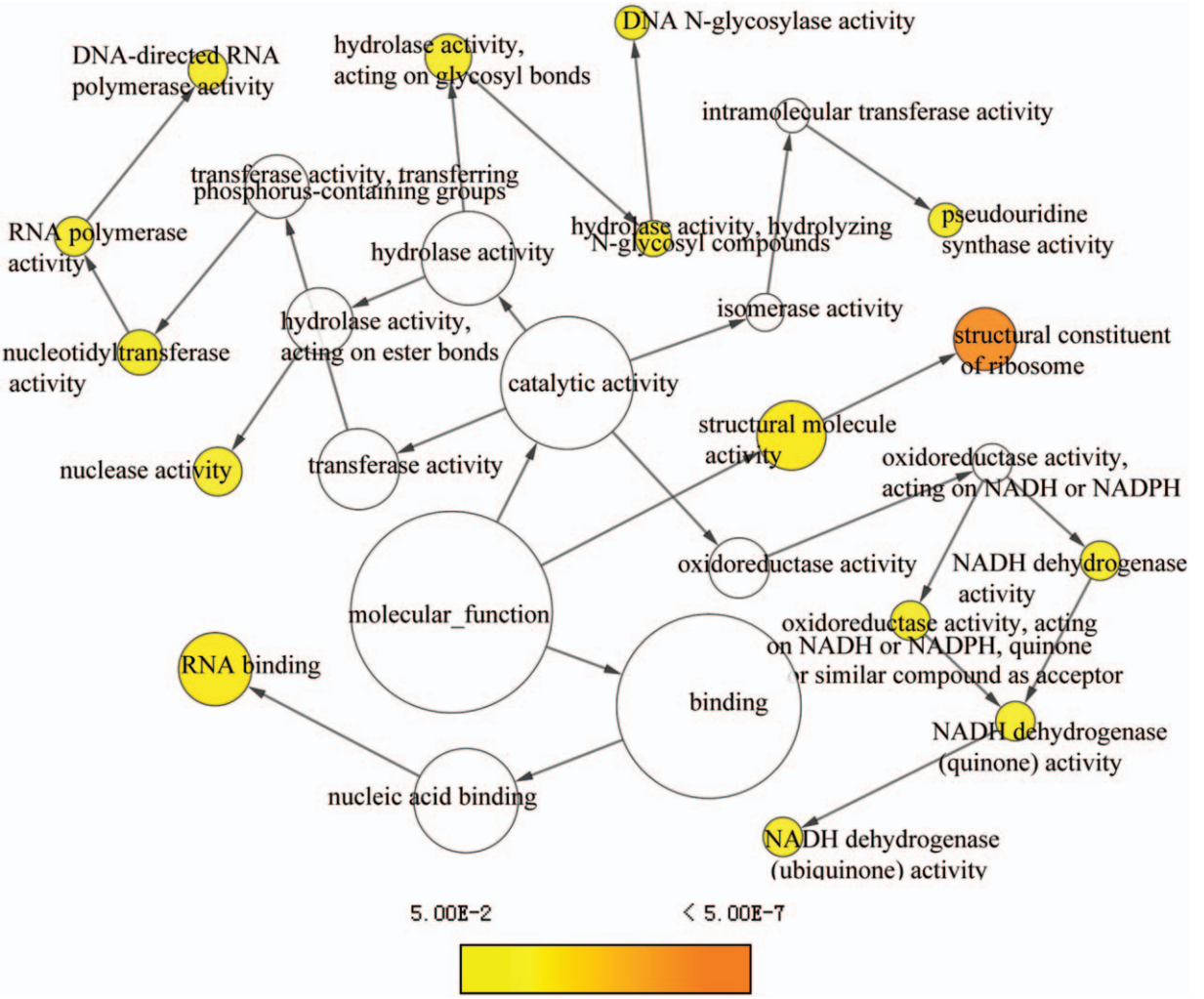

Figure 9. Visualized molecular function results using lncRNA FRGCA-related mRNAs.

and our hypothesis of the association between lncRNA FRGCA and tumor progression in COAD.

Author contributions

Cun Liao and Feng Gao designed this manuscript; Yun Guo, Yizhen Gong, Xue Huang, Xiwen Liao, Xiangkun Wang, and Guotian Ruan conducted the study and analyzed the data. Cun Liao wrote the manuscript and Feng Gao guided the writing and revision.

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