Abstract. In the present study, the significance of GABA_4 genes in colon adenocarcinoma (COAD) were investigated from the view of diagnosis and prognosis. All data were achieved from The Cancer Genome Atlas. Overall survival was analyzed by the Kaplan-Meier analyses and Cox regression model and the hazard ratios and 95% confidence interval were calculated for computation. The Database for Annotation, Visualization and Integrated Discovery, and the Biological Networks Gene Ontology (BiNGO) softwares were applied to assess the biological processes and Kyoto Encyclopedia of Genes and Genomes (KEGG) was used for pathway analysis to predict the biological function of GABA_4 genes. The associated Gene Ontology and KEGG pathways were conducted by Gene Set Enrichment Analysis (GSEA). From receiver operating characteristics curves analysis, it was found that the expression of GABR, γ-aminobutyric acid type A receptor family genes were correlated with COAD occurrence [P<0.0001, area under the curve (AUC)>0.7]. The low expression of the GABRB1, GABRD, GABRP and GABRQ in genes after tumor staging adjustment were positively correlated with the overall survival rate [P=0.049, hazard ratio (HR)=1.517, 95% confidence interval (CI)=1.001-2.297; P=0.006, HR=1.807, 95% CI=1.180-2.765; P=0.005, HR=1.833, 95% CI=1.196-2.810; P=0.034, HR=1.578, 95% CI=1.036-2.405). GSEA showed enrichment of cell matrix adhesion, integrin binding, angiogenesis, endothelial growth factor and endothelial migration regulation in patients with COAD with GABRD overexpression. GABRBI, GABRD, GABRP and GABRQ were associated with the prognostic factors of COAD. The expression levels of GABRBI2, GABRA3, GABRB2, GABRB3, GABRG2, GABRD, GABRE may allow differentiation between tumor tissues and adjacent normal tissues.

Introduction

Colorectal cancer (CRC) is a type of malignant tumor originated from colon and rectum epithelium (1). Most cases of CRC develop slowly through normal mucosal adenoma-cancer sequence for several years and it is one of the most common malignant tumors in the clinic worldwide (2,3). In 2018, the global incidence of colorectal cancer was third from the top among the 36 types of cancer and the mortality rate ranked second and 1.8 million individuals were diagnosed with colorectal cancer in the world (4), the number of deaths due to colorectal cancer was approximately 881,000. Colon cancer is a type of colorectal cancer and accounts for a large proportion of colorectal cancer cases approximately 60.9% in the world in 2018 (4,5). The primary risk factors associated with the disease are elderly, male sex, increased levels of fat consumption, high level of red meat and processed food consumption, lack of exercise, smoking, high alcohol intake (>1 drink/day) (6), obesity and being tall (4,7). The treatment methods of COAD included radiotherapy, surgery, targeted therapy and chemotherapy. Although a great deal of effort has been made to understand the underlying molecular mechanisms of the occurrence and development of COAD, the prevention and treatment of early-onset COAD is still a challenge for researchers (8). Therefore, sensitive and specific biomarkers are needed to improve early diagnosis, aid the management of individualized therapy and predict the prognosis of patients at different stages of the COAD.

γ-Aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the mammalian brain. γ-Aminobutyric
acid type A (GABA<sub>A</sub>) receptors are the primary mediators of inhibitory neurotransmission in the mature brain, which also functions as an agonist-gated ion channel that mediates rapid synaptic inhibition in the mammalian central nervous system (9). The GABA<sub>A</sub> receptor subunit is mainly expressed in the cerebellum and its receptor is located in cerebellum, but GABA<sub>A</sub> is also expressed in testis and CD4-T-cells (10,11). The GABA<sub>A</sub> receptor subunits are a superfamily consisting of 19 subunits: α1-α6 (GABRA1, GABRA2, GABRA3, GABRA4, GABRA5 and GABRA6); β 1-β 3 (GABRB1, GABRB2 and GABRB3); γ 1-γ 3 (GABRG1, GABRG2 and GABRG3); δ (GABRD); ε (GABRE); η (GABRR1); θ (GABRR2); ρ 1-ρ 3 (GABRR1, GABRR2, GABRR3) (9,12,13). However, the data regarding the mRNA expression levels of GABA<sub>A</sub> family genes have not been thoroughly and systematically described. In the present study, the role of the GABA family in colon cancer was studied using the TCGA database. Thus, only 14 genes were analyzed in the present study. Previous study showed that overexpressed GABRD was observed in 89% of cases and had a weak negative correlation with tumor proliferation, proliferative-independent genes are upregulated in tumors and GABA<sub>A</sub> receptors might play a role in the differentiation of tumor cells (14). However, the diagnostic and prognostic value of GABRD and its family members have not been thoroughly and systematically described. In the present study, the role of the GABA family in colon cancer was studied using the TCGA database to obtain survival-associated and GABA<sub>A</sub> family expression in patients with COAD patients and the diagnostic and prognosis value of the mRNA expression levels of GABA<sub>A</sub> family genes were investigated. A few online data portals were applied to analyze functions and signaling pathways to predict the function of these genes.

Materials and methods

Data preparation. The mRNA expression levels and clinical information associated with COAD, including sex, age and tumor-non-metastasis (TNM) stage (8), were obtained from TCGA (cancer.gov/tcga). Overall, 456 patients were performed by mRNA sequencing. The expression data included 480 tumor tissues and 41 adjacent normal tissues. The Bioconductor package (edgeR, version 3.24.3; R, version 3.6.0 software; rstudio, version 1.2.5019) was used to standardize and correct the original data (15). Genes with P-value<0.05 and |log<sub>2</sub> fold-change (FC)<2 were deemed to be significantly different. These genes were regarded as differentially expressed genes (DEGs) (16). First, tumor tissues and adjacent normal tissues data were isolated and then the gene expression data were integrated with clinical information. Finally, patients who had repetition of the data, a survival time of 0 days or no follow-up data were excluded. In the end, 438 tumor tissues and 41 adjacent normal tissues were analyzed in the final research.

mRNA co-expression and functional analysis. In order to analyze the biological pathways and significance of the GABA<sub>A</sub> family genes, a set of functional enrichment analyses were carried out using Database for Annotation, Visualization and Integrated Discovery (DAVID 6.8, david.ncifcrf.gov/home.jsp) (17,18). Enriched P-values <0.05 had statistical significance. These included the terms Gene Ontology (GO) functional examination and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. The functional detection of Molecular functional (MF), cell component (CC) and Biological process (BP) were based on the analysis of GO terminology.

Biological Networks Gene Ontology (BiNGO) (19) was chosen as a tool for GO functional analysis. BiNGO predicted gene function through the consequences of correlation analysis. Gene Multiple Association Network Integration Algorithm (GeneMANIA) was applied for the calculation of the 14 genes of GABA<sub>A</sub> family (20,21). The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database was used to evaluate protein-protein interactions (22) and was applied to evaluate the function and physiological relationships between the GABA<sub>A</sub> family genes. A total score >0.15 was considered to be statistically significant.

Co-representation matrix of GABA<sub>A</sub> families. The correlation between GABA<sub>A</sub> family genes in COAD was determined using Pearson correlation coefficient analysis. An absolute value of correlation coefficient >0.4 was considered strong correlation.

Gene expression level characteristics. Metabolic Gene Rapid Visualizer (MERAV) was performed to create boxplots of the differentially expressed genes of the GABA<sub>A</sub> family in primary colon cancer tissue and normal colon tissue (23). GABA<sub>A</sub> gene expression levels in tumor and adjacent normal tissues were used to construct vertical scatterplots. In addition, the differential expressed genes of the GABA<sub>A</sub> family were screened with the median cut-off values of all genes. Patients who possessed higher value than the median values of GABA<sub>A</sub> genes expression were classified as the high expression group and the other patients were classified into the low expression group.

Diagnostic forecast. GraphPad Prism version 7 (GraphPad Software) was used to construct receiver operating characteristics (ROC) curves to investigate the prognostic value of the GABA<sub>A</sub> genes in patients with COAD in TCGA database. Then the correlation between diagnosis associated genes and tumor stage was investigated using a Spearman’s test and Gene Expression Profiling Interactive Analysis (24). The normalized diagnostic value of P<0.05 was considered to indicate a statistically significant difference.

Survival analysis. According to the median cut-off value of each GABA<sub>A</sub> genes, the patients were categorized into low and high expression groups. P-value and overall survival (OS) of the GABA<sub>A</sub> gene family and clinical data were calculated using Kaplan-Meier analysis and a log-rank test.

To assess the prognostic model thoroughly, a Cox proportional risk regression model for univariate and multivariate survival tests was performed. After adjusting the clinical characteristics, 95% confidence intervals (CIs) and hazard ratios (HRs) were calculated by conducting Cox proportional risk regression model.

Joint-effects analysis. Based on previous survival analysis, joint-effects analysis (25,26) of the prognostic associated genes (GABRB1, GABRD, GABRA5 and GABRR3) was performed to
analyze the effect of polygenes on the survival of patients. Use the following combinations for joint analysis: 1) GABRB1 and GABRD; 2) GABRB1 and GABRP; 3) GABRB1 and GABRQ; 4) GABRD and GABRP; 5) GABRD and GABRQ; 6) GABRP and GABRQ; 7) GABRB1, GABRD and GABRP; 8) GABRB1, GABRD and GABRQ; 9) GABRB1, GABRP and GABRQ; 10) GABRD, GABRP and GABRQ. Each combination was divided into groups based on the median gene expression mentioned earlier (e.g., combination A and B: Group 1=low A+ low B, group 2=low A+ high B or high A+ low B, group 3=high A +high B; combination A, B and C: Group 1=low A+ low B+ low C, group 2=low A+ low B+ high C or low A+ high B+ low C or high A+ low B+ low C, group 3=high A+ high B+ low C or high A+ low B + high C or low A+ high B + high C; group 4=high A+ high B+ high C). According to the above combination, the Cox proportional risk regression model was adjusted for statistical significance factors (i.e., TNM stage). Kaplan-Meier method and log-rank test were used to evaluate the prognostic value of GABA_A genes combination expression in each group.

Nomogram. A nomogram was used to assess the association between GABRB1, GABRD, GABRP, GABRQ and medical rank (gender, age, stage) in terms of OS for patients with COAD. In addition, the potential of these four genes in predicting clinical grade was evaluated.

In terms of clinical data and survival analysis, only tumor stage and GABRB1, GABRD, GABRP and GABRQ expression level entered the risk model after being adjusted by cox proportional hazard regression model. The risk score for all factors were calculated as well as the 1-, 2-, 3-, 4- and 5-year survival rates (27).

Gene set enrichment analysis (GSEA). In order to explore the differences in pathway and biological functions between low- and high-expression groups of the prognostic GABA_A genes, the expression profile of the full-genome dataset in TCGA group was divided into two groups according to the median prognostic GABA_A gene value. GSEA version 3.0 (software.broadinstitute.org/gsea/index.jsp) was applied to explore potential KEGG pathway and GO analysis within the Molecular Signatures Database of c2 curated gene set and c5 GO gene set (28). Criteria for significant enrichment gene sets in GSEA were: P<0.05, False discovery rate <0.25.

Statistical analysis. Statistical analyses were performed using SPSS 20.0 (IBM Corp.) and R version 3.6.0 software. P<0.05 was considered to indicate a statistically significant difference. DAVID was applied to analyze GO and KEGG pathways. The interactive network of the target genes was constructed using Cytoscape version 3.6.1. An unpaired t-test was used to compare data between COAD tumors and adjacent normal tissues. A Spearman's test was performed for the correlation analyses between TNM stages and GABRD expression levels.

Results

Gene expression dataset. Detailed baseline characteristics of 438 patients with COAD patients from TCGA database are summarized in Table I. Sex and age were not associated with OS (all P>0.05), whereas TNM stage was significantly associated with OS (adjusted log-rank test P<0.001).

Biostatistics analysis of GABA_A family genes. The biological functional of the GABA_A genes was investigated using DAVID to evaluate GO functions and KEGG pathways (Fig. 1). Bioinformatics analysis was performed to examine the enrichment of prognostic factors (Table II). The above results indicate that GABA_A genes were involved in the transport of substances and the formation of plasma membrane. In addition, the genes are strongly co-expressed and have complex networks of gene-gene and protein-protein interactions.

Gene expression and diagnostic value of the GABA_A gene family. The vertical scattering map of GABA_A gene expression levels was shown in Fig. 2A, it showed that the results showed that GABRA2, GABRB2, GABRB3 and GABRG2 had low expression in tumor tissues; GABRB1, GABRD, GABRE and GABRP had moderate expression in tumor tissues. The correlation between gene expression and TNM stage showed that the expression levels of GABRD were significantly different in the four tumor stages (I, II, III and IV) from GEPIA (Fig. 2C). In our TCGA database, GABRD expression levels were associated with TNM stage also showed significantly weak positive correlation (Correlation Coefficient=0.174, Table II). The results of MERV showed that the expression levels of GABRA2, GABRA3, GABRB2, GABRB3 and GABRB1 in primary colon tumor tissues were lower compared with normal tissue (Fig. 3A, B, E, F, H and M), whereas the expression levels of GABRA4, GABRB1, GABRG2, GABRD, GABRE, GABRP and GABRG2 in primary colon tumor tissues were higher compared with normal colon tissue (Fig. 3C, D, G, I-L and N). In addition, ROC curves of the predicted expression levels of the GABA_A family genes in tumors and paired colon tissues was constructed (Fig. 3). The expression levels of GABRA2 (Fig. 3A), GABRA3 (Fig. 3B), GABRBB2 (Fig. 3E), GABRB3 (Fig. 3F), GABRG2 (Fig. 3G), GABRG3 (Fig. 3H), GABRD (Fig. 3I) and GABRE (Fig. 3J) were significantly associated with the carcinogenesis of colon tumors (AUC >0.7).

Survival analysis. Univariate survival analysis demonstrated that tumor staging was the only factor associated with OS (P<0.001, Table I). The Kaplan-Meier curve of the GABA_A family genes was presented in Fig. 3A-N. Tumor staging was investigated using Cox proportional hazards regression model for multivariate survival tests, wherein the lower expression levels of GABRB1, GABRD, GABRP and GABRQ were significantly correlated with favorable OS results (adjusted P=0.049, HR=1.517, 95% CI=1.001-2.297; adjusted P=0.006,
Table I. Demographic and clinical data for 438 patients with colon adenocarcinoma.

| Variables     | Patients, n | No. of events\textsuperscript{a} | MST (days) | HR (95% CI) | Log-rank P-value\textsuperscript{b} |
|---------------|-------------|-----------------------------------|------------|-------------|-----------------------------------|
| Sex           |             |                                   |            |             |                                   |
| Male          | 234         | 54                                | 2,475      | 1           | 0.545                             |
| Female        | 204         | 44                                | NA         | 1.131 (0.759-1.686) |                                   |
| Age\textsuperscript{c} (years) |             |                                   |            |             |                                   |
| ≥65           | 168         | 29                                | 2,475      | 1           | 0.114                             |
| <65           | 268         | 116                               | NA         | 1.420 (0.919-2.194) |                                   |
| Tumor stage   |             |                                   |            |             | <0.001\textsuperscript{d}        |
| IV            | 61          | 31                                | 858        | 1           |                                   |
| I             | 73          | 4                                 | NA         | 0.089 (0.031-0.251) |                                   |
| II            | 167         | 27                                | 2,821      | 0.198 (0.018-0.335) |                                   |
| III           | 126         | 31                                | NA         | 0.360 (0.218-0.596) |                                   |

\textsuperscript{a}Number of final events; \textsuperscript{b}Adjusted for tumor stage. \textsuperscript{c}Information of age was unknown in 2 patients. \textsuperscript{d}Information of Tumor-Node-Metastasis stage was not reported in 11 patients; MST, median survival time; CI, confidence interval; HR, hazards ratio; NA, not available.

Figure 1. GO terms and KEGG analysis of all the γ-aminobutyric acid type A family genes using the Database for Explaining, Visualization and Integrated Discovery. GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

HR=1.807, 95% CI 1.180-2.765; adjusted P=0.005, HR=1.833, 95% CI 1.196-2.810 and adjusted P=0.034, HR=1.578, 95% CI 1.036-2.405, respectively; Table III).

The nomogram of scoring risk included the expression levels of GABRB1, GABRD, GABRP and GABRQ and predictive TNM stage, sex, age and 1-, 2-, 3, 4- and 5-year survival
Figure 2. GeneMANIA and STRING analysis of $GABA_A$ genes. (A) Gene interaction networks of $GABA_A$ genes by GeneMANIA. (B) Protein-protein interaction networks of $GABA_A$ genes by STRING. GeneMANIA, Gene Multiple Association Network Integration Algorithm; STRING, search tool for the Retrieval of Interacting Genes/Proteins; $GABA_A$, $\gamma$-Aminobutyric acid type A; $GABR$, $\gamma$-aminobutyric acid type A receptor.

Figure 3. Biological Networks Gene Ontology analysis of $GABA_A$ genes interaction networks. (A) Cellular component outcomes; (B) biological process outcomes; and (C) molecular function outcomes. $GABR$, $\gamma$-aminobutyric acid type A receptor.
Effect of $GABA_A$ genes expression combination on OS. Based on the survival analysis of $GABA_A$ genes, $GABRB1$, $GABRD$, $GABRP$, and $GABRQ$ were selected as prognostic genes by multivariate survival analysis. The joint-effects of these four $GABA_A$ genes on OS in patients with COAD were determined by the joint-effects model. According to the expression levels of $GABRB1$, $GABRD$, $GABRP$, and $GABRQ$, different combinations for this analysis were generated (Tables IV-V). Log-rank tests were performed using Kaplan-Meier analysis to evaluate
the effect of gene expression combinations on the prognosis of patients with COAD (Fig. 9). In the analysis of high expression levels of $GABRB1$, $GABRD$, $GABRP$ and $GABRQ$, the combinations in groups 3, 9, 12, 15, 18, H and P were highly correlated with poor OS (all $P<0.05$; Table VI). Within the evaluation of low $GABRB1$, $GABRD$, $GABRP$ and $GABRQ$ expression levels, the combination of groups 1, 7, 10, 13, 16, A, E, I and M were highly correlated with favorable OS (all $P<0.05$; Table VII).
Figure 6. ROC curves of γ-aminobutyric acid type A genes for distinguishing colon adenocarcinoma tumor tissue and adjacent normal tissues in The Cancer Genome Atlas dataset. ROC curves of: (A) GABRA2; (B) GABRA3; (C) GABRA4; (D) GABRB1; (E) GABRB2; (F) GABRB3; (G) GABRG2; (H) GABRG3; (I) GABRD; (J) GABRE; (K) GABRP; (L) GABRQ; (M) GABRR1; and (N) GABRR2. ROC, Receiver Operating Characteristic. GABR, γ-aminobutyric acid type A receptor; AUC, area under the curve; CI, confidence interval.
GSEA. GSEA of the prognostic genes GABRB1, GABRD, GABRP and GABRQ were performed in the TCGA cohorts (Fig. 10). In the GSEA of KEGG pathways, the expression levels of the GABRD were associated with the chondroitin sulfate...
Table III. Prognostic survival analysis according to high or low expression of γ-aminobutyric acid type A receptor family genes in 438 patients with colon adenocarcinoma.

| Gene     | Patients, n | Events<sup>a</sup> | MST, days | Crude HR (95% CI) | Crude P-value | Adjusted HR (95% CI) | Adjusted P-value<sup>d</sup> |
|----------|-------------|---------------------|-----------|-------------------|---------------|----------------------|-------------------------------|
| GABRA2   | Low         | 219                 | 52        | 2,047             | 1             | 1                    |                               |
|          | High        | 219                 | NA        | 0.869 (0.584-1.292) | 0.487         | 0.792 (0.525-1.196) | 0.267                         |
| GABRA3   | Low         | 219                 | 49        | 3.042             | 1             | 1                    |                               |
|          | High        | 219                 | 49        | 2.532             | 1.088 (0.732-1.618) | 0.675         | 1.099 (0.730-1.654) | 0.651                         |
| GABRA4   | Low         | 219                 | 41        | 2.532             | 1             | 1                    |                               |
|          | High        | 219                 | 57        | 2.047             | 1.530 (1.024-2.287) | 0.038<sup>a</sup> | 1.499 (0.989-2.271) | 0.056                         |
| GABRB1   | Low         | 219                 | 44        | 2.532             | 1.371 (0.920-2.043) | 0.121         | 1.517 (1.001-2.297) | 0.049<sup>a</sup>             |
|          | High        | 219                 | 54        | 1.910             | 1              |                      |                               |
| GABRB2   | Low         | 219                 | 46        | 2.821             | 1              |                      |                               |
|          | High        | 219                 | 52        | 2.475             | 1.108 (0.745-1.647) | 0.614         | 1.343 (0.887-2.033) | 0.163                         |
| GABRB3   | Low         | 219                 | 52        | 2.532             | 0.982 (0.660-1.461) | 0.927         | 1.170 (0.776-1.765) | 0.454                         |
|          | High        | 219                 | 46        | 2.475             | 1.209 (0.809-1.808) | 0.355         | 1.296 (0.854-1.967) | 0.223                         |
| GABRG2   | Low         | 219                 | 51        | 2.821             | 1              |                      |                               |
|          | High        | 219                 | 47        | 2.475             | 1.209 (0.809-1.808) | 0.355         | 1.296 (0.854-1.967) | 0.223                         |
| GABRG3   | Low         | 219                 | 51        | 2.532             | 1              |                      |                               |
|          | High        | 219                 | 47        | 2.475             | 0.971 (0.653-1.445) | 0.886         | 0.958 (0.635-1.445) | 0.839                         |
| GABRD    | Low         | 219                 | 36        | NA                | 1              |                      |                               |
|          | High        | 219                 | 62        | 1.910             | 2.074 (1.374-3.130) | 0.001<sup>b</sup> | 1.807 (1.180-2.765) | 0.006<sup>b</sup>             |
| GABRE    | Low         | 219                 | 57        | 2.134             | 1              |                      |                               |
|          | High        | 219                 | 41        | NA                | 0.744 (0.497-1.111) | 0.149         | 0.736 (0.486-1.112) | 0.145                         |
| GABRP    | Low         | 219                 | 38        | NA                | 1              |                      |                               |
|          | High        | 219                 | 60        | 1.881             | 1.673 (1.113-2.513) | 0.013<sup>a</sup> | 1.833 (1.196-2.810) | 0.005<sup>b</sup>             |
| GABRQ    | Low         | 219                 | 39        | NA                | 1              |                      |                               |
|          | High        | 219                 | 59        | 1.910             | 1.506 (1.005-2.258) | 0.047<sup>a</sup> | 1.578 (1.036-2.405) | 0.034<sup>a</sup>             |
| GABRR1   | Low         | 219                 | 49        | 2.532             | 1              |                      |                               |
|          | High        | 219                 | 49        | 2.134             | 1.070 (0.720-1.591) | 0.736         | 1.079 (0.717-1.625) | 0.714                         |
| GABRR2   | Low         | 219                 | 49        | 3.042             | 1              |                      |                               |
|          | High        | 219                 | 49        | 2.134             | 1.070 (0.720-1.591) | 0.738         | 1.259 (0.833-1.902) | 0.274                         |

<sup>a</sup>P<0.05. <sup>b</sup>P<0.01. <sup>c</sup>Number of final events. <sup>d</sup>Adjusted for tumor stage. HR, hazard ratio; CI, confidence interval; MST, median survival time; GABAA, γ-aminobutyric acid type A.

pathway (Fig. 10H) and GABRP was associated with the intestinal immune network for Immunoglobulin A (IGA) production, hematopoietic cell lineage, the natural killer cell mediated cytotoxicity pathway, sphingolipid metabolism (Fig. 10I-L). GO
function enriched examination demonstrated that that GABRD expression levels were associated with the cell matrix adhesion, integrin, angiogenesis, endothelial growth factor, endothelial migration regulation, and so on (Fig. 10A‑G); whereas GABRB1 and GABRQ had no significant outcomes.

**Discussion**

In the present study, the diagnostic and prognosis value of the GABA$_A$ family genes based on TCGA database were investigated. The results of ROC curves showed that expression levels of GABRB3, GABRG2, GABRD and GABRE had high values to predict the occurrence of colon cancer, among them, GABRD was associated with COAD stage and may have value as an early diagnostic index of COAD. The results were roughly the same as verified in MERA V and Vertical scatterplots. Low expression levels of GABRB1, GABRD, GABRP and GABRQ were associated with favorable COAD OS and the nomogram indicated these four genes had different degrees of influence on the prognosis of the patients, high expression

| Points |   |   |   |   |   |   |   |   |   |   |   |
|--------|--|--|--|--|--|--|--|--|--|--|--|
| Sex    |   |   |   |   |   |   |   |   |   |   |   |
| Male   |   |   |   |   |   |   |   |   |   |   |   |
| Female |   |   |   |   |   |   |   |   |   |   |   |
| age    | ≥65|   |   |   |   |   |   |   |   |   |   |
| <65    |   |   |   |   |   |   |   |   |   |   |   |
| Stage  | I  |   |   |   |   | II |   |   |   | IV |   |
|        |   | I |   |   |   |   | II |   |   |   | IV |
| GABRB1 | High |   |   |   |   |   | High |   |   |   |   |
|        | Low  |   |   |   |   |   | Low  |   |   |   |   |
| GABRD  | High |   |   |   |   |   | High |   |   |   |   |
|        | Low  |   |   |   |   |   | Low  |   |   |   |   |
| GABRP  | High |   |   |   |   |   | High |   |   |   |   |
|        | Low  |   |   |   |   |   | Low  |   |   |   |   |
| GABRQ  | High |   |   |   |   |   | High |   |   |   |   |
|        | Low  |   |   |   |   |   | Low  |   |   |   |   |
| Total Points |   |   |   |   |   |   |   |   |   |   |   |
| 1-year overall survival |   |   |   |   |   |   |   |   |   |   |   |
| 2-year overall survival |   |   |   |   |   |   |   |   |   |   |   |
| 3-year overall survival |   |   |   |   |   |   |   |   |   |   |   |
| 4-year overall survival |   |   |   |   |   |   |   |   |   |   |   |
| 5-year overall survival |   |   |   |   |   |   |   |   |   |   |   |

Figure 8. Nomogram of OS-associated GABRB1, GABRD, GABRP, GABRQ and clinical factors. GABR, γ-aminobutyric acid type A receptor.
Table IV. Grouping according to combination of 2 genes in GABRB1, GABRD, GABRP and GABRQ.

| Group | Combination |
|-------|-------------|
| 1     | Low GABRB1 + Low GABRD |
| 2     | Low GABRB1 + High GABRD |
| 3     | High GABRB1 + Low GABRD |
| 4     | Low GABRB1 + Low GABRP |
| 5     | Low GABRB1 + High GABRP |
| 6     | High GABRB1 + Low GABRP |
| 7     | Low GABRB1 + Low GABRQ |
| 8     | Low GABRB1 + High GABRQ |
| 9     | High GABRB1 + Low GABRQ |
| 10    | Low GABRB1 + Low GABRP |
| 11    | Low GABRD + High GABRP |
| 12    | High GABRD + High GABRP |
| 13    | Low GABRD + Low GABRP |
| 14    | Low GABRD + High GABRP |
| 15    | High GABRD + High GABRP |
| 16    | Low GABRP + Low GABRQ |
| 17    | Low GABRP + High GABRP |
| 18    | High GABRP + High GABRP |

GABR, γ-aminobutyric acid type A receptor.

of GABRB1, GABRD, GABRP have high contribution to the risk score than high expression of GABRQ. In the functional evaluation of GO and KEGG, it was found that the functions of the GABA_A family gene were significantly enriched in cell junction, integral component of membrane, signal transduction, integral component of plasma membrane.

GABA_A receptors have the same structure with nicotinic acetylcholine receptors, the 5-hydroxytryptamine type 3 receptor and zinc-activated channel, all with pentameric structures and belonging to the agonist-gated ion channel superfamily (29). STRING results showed that obvious gene fusions, gene co-occurrence and co-expression between GABA_A genes. Pearson correlation coefficient analysis showed that there was a correlation between the expression levels of some genes in the GABA_A family, especially between GABRB1 and GABA4, and GABRQ and GABRG2.

The GABA_A family gene also serves a role in several types of cancer. Gumireddy et al (30) found that the high expression levels of GABRA3 were inversely proportional to the survival rate of patients with breast cancer and that GABRA3 activated the AKT pathway which promoted the migration, invasion and metastasis of breast cancer cells. Therefore, GABRA4 might serve a role in COAD, which requires further study. Bautista et al (31) observed that the expression levels of GABRA6 in tumor initiating stem cells (TISCS) and hepatocellular carcinoma (HCC) were reduced, whereas the expression levels of GABRG3 were abundant in TISCS and limited in HCC. A previous study showed that the specific activation of GABA_A receptor decreased cell activity, induced apoptosis and inhibited the growth and survival signal pathway of neuroblastoma cells (32). Chen et al (33) found that GABA_A receptor could inhibit the migration and invasion of human hepatocellular carcinoma cells and Minuk et al (34) reported downregulated expression of the GABRB3 receptor in liver tissue of human hepatocellular carcinoma, which was consistent with COAD in the present study. Takehara et al (35) found that GABA promoted the growth of pancreatic cancer by expressing GABA_A receptor GABRP subunit. Zhang et al showed that RNA binding protein nova 1 and GABRG2 interacted in the central nervous system and in liver cancer. Nova 1, as a potential mechanism of oncogene, might interact with GABRG2 (36). To sum up, the GABA_A family plays an important role in many cancer types. Nevertheless, the correlation between GABA_A family and COAD is unclear. Here, we use the TCGA database to study the correlation of GABAA gene family expression with diagnosis and prognosis.

GSEA analysis showed that GABRD was associated with cell matrix adhesion and integrin binding. Cell adhesion is an important cellular process that could lead to cancer (37,38). As the main receptor of cell matrix adhesion, integrin exists on the surface of tumor and stroma cells, which had a profound impact on cancer cell's ability to survive in a specific location, cell adhesion and integrin can worked together to lead to apoptosis (39). In addition, integrin also serves a role in promoting the phenotype of tumor cells (40). The present study also suggested that GABRD was significantly associated with angiogenesis and endothelial migration regulation in GSEA. These factors serve a role in tumor invasion and migration (41-43). In addition, tumor angiogenesis is also one of the markers of tumor progression and the increase of tumor microvessel density is an index of poor prognosis (44). Park et al reported that human γ-aminobutyrate type A receptor-binding protein (GABARB) could inhibit angiogenesis by directly binding to vascular endothelial growth factor receptor 2 (VEGFR-2) to inhibit the phosphorylation of PI3K/AKT pathway related proteins (45). GABARBP served a role in regulating the activity of GABA_A receptor, a key participant in intracellular trafficking in all the GABA_A receptors (46-48). Therefore, the GABA_A family genes may affect angiogenesis through regulating GABARBP, which needs to be verified in future experiments. In the present study, KEGG pathway analysis showed that GABRD was associated with chondroitin sulfate synthesis. Chondroitin sulfate serves a role in cancer metastasis and chondroitin sulfate-E negatively adjusted breast cancer cell motility through the Wnt/β-catenin-Collagen I axis (49,50).

In the present study, it was observed that the expression of GABRD mRNA in adjacent tissues was significantly lower compared with COAD tumor tissues, which was consistent with the results of a previous study (14). KEGG pathway analysis of the present study showed that GABRD was associated with intestinal immune network for IGA production, hematopoietic cell lineage, natural killer (NK) cell mediated cytotoxicity and sphingolipid metabolism. In previous studies, people with IgA deficiency were found to have a moderately increased risk of
### Table V. Grouping according to combination of 3 genes in GABRB1, GABRD, GABRP and GABRQ.

| Group | Combination                                      | Group | Combination                                      |
|-------|-------------------------------------------------|-------|-------------------------------------------------|
| A     | Low GABRB1 + Low GABRD + Low GABRP             | I     | Low GABRB1 + Low GABRP + Low GABRQ             |
| B     | Low GABRB1 + High GABRD + Low GABRP            | J     | Low GABRB1 + High GABRP + Low GABRQ            |
|       | Low GABRB1 + Low GABRD + High GABRP            | K     | High GABRB1 + Low GABRP + Low GABRQ            |
|       | High GABRB1 + Low GABRD + Low GABRP            | L     | High GABRB1 + High GABRP + Low GABRQ           |
| C     | High GABRB1 + High GABRD + Low GABRP           | M     | Low GABRD + Low GABRP + Low GABRQ             |
|       | High GABRB1 + Low GABRD + High GABRP           | N     | Low GABRD + High GABRP + Low GABRQ            |
| D     | High GABRB1 + High GABRD + High GABRP          | O     | High GABRD + High GABRP + Low GABRQ           |
| E     | Low GABRB1 + Low GABRD + Low GABRQ             | P     | High GABRD + High GABRP + High GABRQ          |
| F     | Low GABRB1 + High GABRD + Low GABRQ            |       |                                                 |
|       | Low GABRB1 + Low GABRD + High GABRP            |       |                                                 |
|       | High GABRB1 + Low GABRD + Low GABRQ            |       |                                                 |
| G     | High GABRB1 + High GABRD + Low GABRP           |       |                                                 |
| H     | High GABRB1 + High GABRD + High GABRP          |       |                                                 |

GABR, γ-aminobutyric acid type A receptor; 1-18, 2 selected genes groups; A-P, 3 selected genes groups.

---

**Figure 9.** Survival curves for joint-effects analysis of the combination of GABAA genes in patients with colon adenocarcinoma in TCGA dataset. Joint-effects analysis of (A) GABRB1 and GABRD; (B) GABRB1 and GABRP; (C) GABRB1 and GABRQ; (D) GABRD and GABRP; (E) GABRD and GABRQ; (F) GABRP and GABRQ; (G) GABRB1, GABRD and GABRP; (H) GABRB1, GABRD and GABRQ; (I) GABRB1, GABRP and GABRQ; (J) GABRD, GABRP and GABRQ. GABR, γ-aminobutyric acid type A receptor; TCGA, the Cancer Genome Atlas.
cancer, especially gastrointestinal cancer (51). NK cells also play an important role in mediating immune surveillance for human cancer (52). As the structural molecules of cell membranes, sphingolipids play an important role in maintaining barrier

Table VI. Joint analysis of the prognostic value of 2-gene combinations in GABRB1, GABRD, GABRP and GABRQ expression of patients with colon adenocarcinoma.

| Group | Patients | MST, days | Crude P-value | Crude HR | Adjusted P-value | Adjusted HR (95% CI)¹ |
|-------|----------|-----------|---------------|----------|------------------|-----------------------|
| 1     | 115      | 1         | 0.003ᵇ        | 1        | 0.007ᵇ          | 1                     |
| 2     | 208      | 2,821     | 0.021ᵃ        | 1.947 (1.105-3.431) | 0.020ᵃ        | 2.009 (1.118-3.611) |
| 3     | 115      | 1,849     | 0.001ᵇ        | 2.814 (1.551-5.104) | 0.002ᵇ        | 2.712 (1.460-5.039) |
| 4     | 112      | 2,134     | 0.985         | 1        | 0.921           | 1                     |
| 5     | 214      | 3,042     | 0.865         | 1.042 (0.648-1.676) | 0.966         | 1.011 (0.616-1.659) |
| 6     | 112      | 1         | 0.947         | 1.019 (0.587-1.768) | 0.720         | 1.110 (0.628-1.962) |
| 7     | 110      | 1         | 0.024ᵃ        | 1        | 0.011ᵃ          | 1                     |
| 8     | 218      | 2,821     | 0.506         | 1.200 (0.702-2.051) | 0.263         | 1.381 (0.784-2.431) |
| 9     | 110      | 1,711     | 0.016ᵃ        | 1.994 (1.137-3.497) | 0.005ᵇ        | 2.333 (1.287-4.231) |
| 10    | 112      | 1         | 0.000ᶜ        | 1        | 0.006ᵇ          | 1                     |
| 11    | 214      | 2,532     | 0.001ᵇ        | 2.936 (1.530-5.634) | 0.006ᵇ        | 2.620 (1.318-5.207) |
| 12    | 112      | 1,849     | 0.000ᵇ        | 4.026 (2.042-7.937) | 0.000ᵇ        | 4.033 (1.967-8.270) |
| 13    | 110      | 3,042     | 0.000         | 1        | 0.001ᵇ          | 1                     |
| 14    | 218      | 1         | 0.249         | 1.402 (0.790-2.490) | 0.332         | 1.342 (0.741-2.431) |
| 15    | 110      | 1,493     | 0.000ᶜ        | 2.934 (1.639-5.255) | 0.002ᵇ        | 2.658 (1.453-4.863) |
| 16    | 110      | 1         | 0.001ᵇ        | 1        | 0.000ᶜ          | 1                     |
| 17    | 218      | 1         | 0.332         | 1.342 (0.741-2.431) | 0.249         | 1.402 (0.790-2.490) |
| 18    | 110      | 1,661     | 0.000ᵇ        | 2.658 (1.453-4.863) | 0.000ᶜ        | 2.934 (1.639-5.255) |

ᵃP<0.05,ᵇP<0.01,ᶜP<0.001. ¹1-18, 2 selected genes groups. ÑAdjustment for TNM stage. GABR, γ-aminobutyric acid type A receptor; MST, median survival time; HR, hazard ratio; CI, confidence interval; GABR, γ-aminobutyric acid type A receptor.

Table VII. Joint analysis of the prognostic value of 3 genes combination in GABRB1, GABRD, GABRP and GABRQ expression of patients with colon adenocarcinoma.

| Group | Patients | MST, days | Crude P-value | Crude HR | Adjusted P-value | Adjusted HR (95% CI)¹ |
|-------|----------|-----------|---------------|----------|------------------|-----------------------|
| A     | 61       | 1         | 0.001ᵇ        | 1        | 0.000ᶜ          | 1                     |
| B     | 148      | 3,042     | 0.000         | 0.130 (0.044-0.386) | 0.000ᵇ        | 0.103 (0.030-0.357) |
| C     | 178      | 2,047     | 0.007ᵇ        | 0.439 (0.241-0.801) | 0.002ᵇ        | 0.374 (0.201-0.695) |
| D     | 51       | 1,849     | 0.127         | 0.649 (0.373-1.131) | 0.060         | 0.581 (0.330-1.023) |
| E     | 57       | 1         | 0.001ᵇ        | 1        | 0.006ᶜ          | 1                     |
| F     | 164      | 3,042     | 0.864         | 1.072 (0.487-2.359) | 0.945         | 0.971 (0.418-2.255) |
| G     | 158      | 2,134     | 0.047ᵃ        | 2.159 (1.011-4.607) | 0.030ᵃ        | 2.439 (1.089-5.462) |
| H     | 59       | 1,348     | 0.007ᵇ        | 3.067 (1.365-6.892) | 0.028ᵇ        | 2.626 (1.110-6.231) |
| I     | 52       | 1         | 0.002ᵇ        | 1        | 0.000ᶜ          | 1                     |
| J     | 168      | 1         | 0.015ᵃ        | 0.360 (0.157-0.823) | 0.003ᵇ        | 0.249 (0.099-0.629) |
| K     | 165      | 1,881     | 0.001ᵇ        | 0.349 (0.193-0.632) | 0.000ᵇ        | 0.304 (0.166-0.556) |
| L     | 53       | 1,503     | 0.119         | 0.651 (0.380-1.116) | 0.046         | 0.573 (0.332-0.989) |
| M     | 59       | 3,042     | 0.000ᶜ        | 1        | 0.000ᶜ          | 1                     |
| N     | 155      | 1         | 0.250         | 1.685 (0.693-4.096) | 0.106         | 2.222 (0.845-5.843) |
| O     | 170      | 1,881     | 0.014ᵃ        | 2.914 (1.240-6.852) | 0.026ᵃ        | 2.883 (1.136-7.318) |
| P     | 54       | 1,381     | 0.000ᶜ        | 5.003 (2.034-12.307) | 0.000ᶜ        | 7.157 (2.689-19.053) |

ᵃP<0.05,ᵇP<0.01,ᶜP<0.001. ¹Adjustment for TNM stage. GABR, γ-aminobutyric acid type A receptor; MST, median survival time; HR, hazard ratio; CI, confidence interval.
Besim considered that signaling nodes in sphingolipid metabolism, such as sphingolipids, metabolic enzymes, and/or receptors, are new therapeutic targets for the development of new anticancer intervention strategies.

At present, few reports have been reported on GABRB1 in tumor field, our present study showed that GABRB1 was differentially expressed in tumor and adjacent normal tissues and that high expression levels of GABRB1 in patients with COAD was associated with a less favorable OS. Hence, GABRB1 may also have potential as a prognosis biomarker of COAD.

In previous studies, GABRD was upregulated in patients with COAD and was not associated with proliferation, which is consistent with the results of the present study. Sarathi et al found GABRD was significantly monotonically upregulated across stages in hepatocellular carcinoma. In the present study, it was demonstrated that the expression of GABRD in COAD was significantly upregulated compared with normal tissues. Low expression levels of GABRD were associated with a more favorable prognosis and could be used as a biomarker for prognosis.

At present, it is known that GABRP serves a role in cancer development and progression. Menelaos et al found that GABRP gradually downregulated as tumors progressed, and it may serve as a prognostic marker for breast cancer. In contrast, Symmans et al found increased expression of GABRP gene in undifferentiated cell type breast cancer and is significantly associated with shorter lifetime history of breastfeeding and with high-grade breast cancer in Hispanic women. Sung et al found that GABRP enhances aggressive phenotype of ovarian cancer cells. Jiang et al found that the expression of GABRP in pancreatic cancer tissues was significantly increased and associated with poor prognosis, contributing to tumor growth and metastasis. In our study, we found that the expression of GABRP in cancer tissues was higher than in adjacent normal tissues. Low expression levels of GABRP were associated with more favorable prognosis and could be used as a biomarker for prognosis.

There were some limitations in the present study. First, the sample size was relatively small. Second, the clinical data were slightly inadequate, such as Event-free Survival (EFS) information, smoking, drinking history, tumor size and lymph node
metastasis were not available from TCGA database. Therefore, it was not possible to perform a far-reaching survival analysis of GABA$_A$ genes considering each potential prognostic variable of COAD in the multivariate Cox proportional hazards regression model. Third, although the association between the GABA$_A$ gene family mRNA levels and COAD prognosis was investigated, the association between GABA$_A$ family protein levels and COAD, GABA$_B$ genes and GSEA still require further experimental research. Experiments like cell migration assays, detection of sulfonic acid related pathways at protein level and the functions of these genes in common cancer-related pathways, such as PI3K/AKT signaling pathway (61), JAK/STAT signaling pathway (62), should be conducted in future. However, despite these limitations, the present study further showed that the downregulated expression levels of GABRB1, GABRD, GABRP and GABRQ in COAD was associated with a more favorable prognosis and the potential mechanisms of GSEA associated with to GABRD and GABRP in the prognosis of COAD were studied. These results need to be verified with a larger sample size to confirm the role of the GABA$_A$ family genes in the diagnosis and prognosis of COAD in the future.

Overall, the present study showed that the upregulated expression levels of GABRA2, GABRA3, GABRB2, GABRB3, GABRG2, GABRG3, GABRD and GABRE in COAD may have potential diagnostic value in COAD. In addition, the low expression levels of GABRB1, GABRD, GABRP and GABRQ were associated with a more favorable prognosis of patients with COAD and could be used as a prognostic biomarker. Multivariate survival analysis, nomograms and joint survival analysis showed that the high expression of GABRB1, GABRD, GABRP and GABRQ were associated with poor prognosis of COAD. GSEA suggested that GABRD may impact cell adhesion, integrin binding, angiogenesis and so on; GABRP was associated with intestinal immune network for IGA production, hematopoietic cell lineage, and so on. However, the results of the present need to be confirmed by further research.

Acknowledgements

The authors thank the contributors of The Cancer Genome Atlas (portal.gdc.cancer.gov/) and proteinatlas.org for their contribution to share the colon adenocarcinoma dataset on open access.

Funding

The present study was supported by the Innovation Project of Guangxi Graduate Education (grant no. JGY2019052) and Self-financing Scientific Research Project of Guangxi Zhuang Autonomous Region Health Commission, China (grant no. Z20180959).

Availability of data and materials

The analyzed datasets generated during the study are available in The Cancer Genome Atlas repository (cancer.gov/tcga).

Authors’ contributions

LY, MS and JG conceived and designed the study. XL, XW, QH, YG, HX and GR processed the data and performed the statistical analysis and they also generated and modified the figures. LY, LZ, XZ and FG wrote and revised the manuscript and helped to perform the analysis and interpretation of data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Fessler E and Medema JP: Colorectal cancer subtypes: Developmental origin and microenvironmental regulation. Trends Cancer 2: 505-518, 2016.
2. Takayama T, Ohi M, Hayashi T, Miyashita K, Nobeoka A, Nakajima T, Satoh T, Takimoto R, Kato J, Sakamaki S and Nishio Y: Analysis of K-ras, APC, and beta-catenin in aberrant crypt foci in sporadic adenoma, cancer, and familial adenomatous polyposis. Gastroenterology 121: 599-611, 2001.
3. Juspeert JE, Vermeulen L, Meijer GA and Dekker E: Serrated neoplasia-role in colorectal carcinogenesis and clinical implications. Nat Rev Gastroenterol Hepatol 12: 401-409, 2015.
4. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394-424, 2018.
5. Labianca R, Beretta GD, Kildani B, Milesi L, Merlin F, Mosconi S, Pessi MA, Prochilo T, Quadri A, Gatta G, et al: Colon cancer. Crit Rev Oncol Hematol 74: 106-133, 2010.
6. Fedirko V, Tramacere I, Bagnardi V, Rota M, Scotti L, Islami F, Negri E, Straif K, Romieu I, La Vecchia C, et al: Alcohol drinking and colorectal cancer risk: An overall and dose-response meta-analysis of published studies. Ann Oncol 22: 1958-1972, 2011.
7. Campbell PT, Cotterchio M, Dick E, Parfrey P, Gallinger S and Neelands TR and Macdonald RL: Incorporation of the pi subunit to human genomics. The human transcriptome evolution of the GABA(A) receptor gene family. Cell Mol Neurobiol 25: 607-624, 2005.
8. Mele M, Ferreira PG, Reverter F, DeLuca DS, Monlongj J, Sannet M, Young TR, Goldmian JM, Pervouchine DD, Sullivan TJ, et al: Human genomics. The human transcriptome across tissues and individuals. Science 348: 660-665, 2015.
9. Tian J, Lu Y, Zhang H, Chau CH, Dang HN and Kaufman DL: Gamma-aminobutyric acid inhibits T cell autoimmunity and the development of inflammatory responses in a mouse type 1 diabetes model. J Immunol 173: 5298-5304, 2004.
10. Macdonald RL and Olsen RW: GABA(A) receptor channels. Annu Rev Neurosci 17: 569-602, 1994.
11. Nelson TR and Macdonald RL: Incorporation of the pi subunit into functional gamma-aminobutyric Acid(A) receptors. Mol Pharmacol 56: 598-610, 1999.
12. Gross AM, Kreisberg JF and Iedeker T: Analysis of matched tumor and normal profiles reveals common transcriptional and epigenetic signals shared across cancer types. PLoS One 10: e0142618, 2015.
13. Robinson MD, McCarthy DJ and Smyth GK: Edger: A bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26: 139-140, 2010.
14. Mourir M, Lacchetta M, Silva TC, Olsen C, Bontempi G, Chen X, Noushmehr H, Colaprico A and Papaleo E: New functionalities in the TCGAbiolinks package for the study and integration of cancer data from GDC and GTEx. PLoS Comput Biol 15: e1006701, 2019.
17. Huang da W, Sherman BT and Lempicki RA: Bioinformatics enrichment tools: Paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 37: 1-13, 2009.

18. Huang da W, Sherman BT and Lempicki RA: Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4: 44-57, 2009.

19. Maere S, Heymans K and Kuiper M: BiNGO: A cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. Bioinformatics 21: 3446-3448, 2005.

20. Szklarczyk D, Franceschini A, Kuhn M, Wyder S, blindsle M, Zollinger L, Gable A, Wu B, Los Arcos C, Donaldson SL, Morris Q and Bader GD: GeneMANIA cyto-structure of the native GABA(A) receptor determined by electron microscopy analysis of large gene lists. Nucleic Acids Res 37: 1-13, 2009.

21. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES and Mesirov JP: Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA 102: 15545-15550, 2005.

22. Nayernia N, Green TP, Martin H and Bader ED: Quaternary structure of the native GABAB receptor determined by electron microscopic image analysis. J Neurochem 62: 815-818, 1994.

23. Guimiredy K, Li A, Kosenkova AV, Sakurai M, Yan J, Li Y, Xu H, Wang J, Zhang PJ, Zhang L, et al: The mRNA-edited form of GABRA3 suppresses GABRA3-mediated Akt activation and breast cancer cell growth. Cancer Biol Med 9: 90-98, 2012.

24. Bautista W, Perez-Alvarez V, Burczynski F, Raouf A, Klonisch T and Minuk G: Membrane potential differences and GABAB receptor expression in hepatic tumor and non-tumor stem cells. Can J Physiol Pharmacol 92: 85-91, 2014.

25. Hackett CS, Quigley DA, Wong RA, Chen J, Cheng C, Song YK, Wei JS, Pavlikowska L, Bao Y, Goldenberg DP, et al: Expression quantitative trait loci and receptor pharmacology implicate Arg1 in the GABA-A receptor as therapeutic targets in neuroblas-toma. Cell Rep 9: 1034-1046, 2014.

26. Chen ZA, Bao MY, Xu YE, Zha RP, Shi HB, Chen TY and He XH: Suppression of liver cancer cell migration and invasion via the GABA(A) receptor. Cancer Biol Med 9: 90-98, 2012.

27. Minuk KY, Zhang M, Gong Y, Minuk L, Dienes H, Pettigrew N, Kew M, Lipkin JS, Takahashi T, et al: Decreased hepatocyte membrane receptor expression in hepatic tumor and non-tumor stem cells. Int J Cancer 118: 1453-1459, 2006.

28. Okumura T, Zhou RP, Shi HB, Chen TY and He XH: The role of cell adhesion molecule in cancer progression and its application in cancer therapy. Acta Biochim Pol 51: 445-457, 2004.

29. Kawcich T: Cell adhesion and its endocytic regulation in cell migration during neural development and cancer metastasis. Int J Mol Sci 13: 4564-4590, 2012.

30. Desgrother JS and Cheung K: Integrins in cancer: Biological implications and therapeutic opportunities. Nat Rev Cancer 10: 9-22, 2010.

31. Mouw JK, Yui Y, Damiano L, Bainer RO, Laksins JN, Aberci I, Ou G, Wijkekoen AC, Levental KR, Gilbert PM, et al: Tissue mechanics modulate microRNA-dependent PTEN expression to regulate malignant progression. Nat Med 20: 360-367, 2014.

32. Kimura C, Bhat SM, Zhao L, Zhang Z and Oke M: Endothelium-dependent epithelial-mesenchymal transition of tumor cells: Exclusive roles of transforming growth factor β1 and β2. Biochim Biophys Acta 1830: 4470-4481, 2013.

33. Ghesquiere B, Wong BW, Kuchnio A and Carmeliet P: Metastasis: The role of cell adhesion and immune cells in health and disease. Nature 511: 167-171, 2014.

34. Lee E, Panedy NB and Popel AS: Crosstalk between cancer cells and blood endothelial and lymphatic endothelial cells in tumour and organ microenvironment. Expert Rev Mol Med 17: e3, 2015.

35. Hanahan D and Weinberg RA: Hallmarks of cancer: The next generation. Cell 144: 646-674, 2011.

36. Park SH, Kim BR, Lee JH, Park ST, Lee SH, Dong SM and Rho SB: GABARBP down-regulates HIF-1α expression through the VEGF-R2 and PI3K/mTOR/4E-BP1 pathways. Cell Signal 26: 1506-1513, 2014.

37. Osumi Y: Molecular dissection of autophagy: Two ubiquitin-like systems. Nat Rev Mol Cell Biol 2: 211-216, 2001.

38. Mizushima N: The pleiotropic role of autophagy: From protein metabolism to bactericide. Cell Death Differ 12 (Suppl 2): S155-S154, 2005.

39. Zhu JH, Horbinski C, Guo F, Watkins S, Uchiyama Y and Chu CT: Regulation of autophagy: The GABRB5 and GABRA1 signal-regulated protein kinases during l-methyl-4-phenylpyridinium-induced cell death. Am J Pathol 170: 75-86, 2007.

40. Mizumoto S, Yamada S and Sugahara K: Molecular interactions between chondroitin-dermatan sulfate and growth factors/recep-tor-matrix proteins. Curr Opin Struct Biol 14: 35-42, 2005.

41. Willis CM and Kluppel M: Chondroitin sulfate-E is a negative regulator of a pro-tumorigenic Wnt/beta-catenin-Collagen 1 axis in breast cancer cells. PLOS One 9: e103966, 2014.

42. Ludvigsson JF, Neovius M, Ye W and Hammarstrom L: IgA deficiency and risk of cancer: A population-based matched cohort study. J Clin Immunol 35: 182-188, 2015.

43. Malmberg JG, Carlsten M, Bjorklund A, Solberg E, Bryceons YT and Ljunggren HG: Natural killer cell-mediated immunosurveil-lance of human cancer. Semin Immunol 31: 20-29, 2017.

44. Hanum VA and Obeid LM: Principles of bioactive lipid signalling: Lessons from sphingolipids. Nat Rev Mol Cell Biol 19: 139-150, 2008.

45. Ogrepimen B: Sphingolipid metabolism in cancer signalling and therapy. Nat Rev Cancer 18: 33-50, 2018.

46. Sarathi A and Palapinniyan A: Novel significant stage-specific differentially expressed genes in hepatocellular carcinoma. BMC Cancer 19: 663, 2019.

47. Kozlov M, Chu JM, Bouchard S and Ljunggren HG: Natural killer cell-mediated immunosurveillance of human cancer. Expert Rev Mol Med 17: e3, 2015.

48. Park SJ, Park JH, Lee SH and Hsieh JT: The role of cell adhesion and immune cells in health and disease. Nature 511: 167-171, 2014.