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Original research

Enhanced expression of GABRD predicts poor prognosis in patients with colon adenocarcinoma

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ABSTRACT

Neurotransmitters are reported to be involved in tumor initiation and progression. This study aimed to elucidate the prognostic value of γ-aminobutyric acid type A receptor δ subunit (GABRD) in colon adenocarcinoma (COAD) using the data from The Cancer Genome Atlas (TCGA) database. The GABRD mRNA expression levels in the COAD and normal tissues were compared using the Wilcoxon rank-sum test. The correlation between clinicopathologic characteristics and GABRD expression was analyzed by Wilcoxon rank-sum test or Kruskal-Wallis test and logistic regression. The prognostic value of GABRD mRNA expression in patients with COAD was determined using the Kaplan-Meier curve and Cox regression analysis. Finally, the molecular mechanisms of GABRD in COAD were predicted by gene set enrichment analysis (GSEA). The COAD tissues exhibited higher GABRD mRNA expression levels than the normal tissues. The logistic regression analysis revealed that GABRD mRNA expression was correlated with TNM stage, N stage, M stage, and microsatellite instability (MSI) status. The Kaplan-Meier survival curve and log-rank test revealed that patients with COAD exhibiting high GABRD mRNA expression were associated with poor overall survival (OS). The multivariate analysis indicated that increased GABRD mRNA expression was an independent prognostic factor and was correlated with a poor OS. The GSEA revealed that GABRD was involved in signaling pathways, including cell adhesion molecules, gap junction, melanogenesis, and mTOR signaling pathway, as well as the signaling pathways associated with basal cell carcinoma or bladder cancer development. In summary, enhanced GABRD mRNA expression may be a potential independent prognostic biomarker for COAD.

Introduction

Globally, colon cancer is one of the most common malignancies and the most frequent cause of cancer-related death. An estimated 1.1 million new cases of colon cancer were diagnosed and 551,000 deaths were recorded in 2018 [1]. The most common pathological type of colon cancer is colon adenocarcinoma (COAD), which accounts for more than 90% of colon cancer cases [2]. Patients with COAD exhibit varied responses to therapy due to the heterogeneity of COAD [3]. It is important to identify reliable prognostic biomarkers as they can potentially distinguish high-risk patients who must be considered for further therapy. Additionally, reliable prognostic biomarkers can identify low-risk patients for whom observation is a prudent approach and can aid in avoiding potentially toxic cancer treatment [4]. Currently, the prognosis of COAD is mainly determined based on TNM staging and other clinicopathologic characteristics. However, there is a marked variation in the survival outcomes of patients with the same stage tumor who are subjected to anti-cancer therapy based on these traditional prognostic biomarkers. Hence, these traditional biomarkers have limited prognostic value [5]. In the past few decades, several studies have identified various predictive biomarkers, such as *RAS* mutation status, *BRAF* mutation status, and microsatellite instability (MSI) status, which can aid in identifying patients at high risk of tumor progression or recurrence [6,7]. Recently a set of novel prognostic biomarkers, such as immunoscore and tumor budding have been identified. Tumors can be classified as low, intermediate and high immunoscore, of which low immunoscore cancer patients have been non-infiltrated by CD3+ and CD8+ lymphocytes and placed at risk [8]. Tumor budding is an important negative prognostic feature, which is strongly associated with lymph node metastasis, recurrence and cancer-related death in colorectal cancer [9]. Although existing biomarkers are commonly used for predicting long-term

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outcome, some patients are still misdiagnosed [10]. Therefore, it is necessary to identify novel biomarkers that can be used to predict prognosis for COAD.

The tumor microenvironment is reported to play a critical role in tumor progression [11,12]. Similar to the processes of angiogenesis and lymphangiogenesis, some studies have reported that tumors are associated with neoneurogenesis, a process that involves the infiltration of new growing nerve endings into the tumor [13,14]. Recent studies have suggested that nerves infiltrate the tumor microenvironment and stimulate cancer cell growth and metastasis through the neurotransmitter-initiated signaling pathway [15,16]. Additionally, the neurotransmitters in the tumor microenvironment can affect the immune cells, endothelial cells, and stromal cells to promote tumor progression by binding to the corresponding neurotransmitter receptors [17]. γ-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the adult mammalian central nervous system. GABA receptors, which are expressed in various tumor tissues, exert regulatory effects on tumor cell proliferation and migration [18–21]. There are two main classes of GABA receptors: ionotropic ($GABA_A$ and $GABA_C$ receptor) and metabotropic ($GABA_B$) receptors [22]. Generally, GABA stimulates tumor cell proliferation and migration through the $GABA_A$ receptor whose expression is enhanced in breast cancer, pancreatic cancer, prostate cancer, and liver cancer [23–26]. Furthermore, the overexpression of the $GABA_A$ receptor is reported to promote the proliferation of gastric cancer cell line (KATO III) by activating the ERK-1/2/cyclin D1 pathway [27]. The neoplastic tissues of colorectal cancer are reported to exhibit enhanced GABA contents and upregulated $GABA_A$ receptor expression [28]. However, the mechanisms underlying the role of $GABA_A$ receptors in colorectal cancer are not elucidated. $GABA_A$ receptors are ligand-gated chloride channels, which comprise 19 different subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π, and ρ1–3) [20]. GABRD, the δ subunit of the $GABA_A$ receptor, is encoded in the human chromosome 1p36 region, whose involvement in cancers is not fully elucidated. Recent studies have demonstrated that GABRD expression may serve as an independent prognostic marker for one subtype of glioma [29]. Additionally, the *GABRD* gene is reported to be a stage-specific differentially expressed gene in hepatocellular carcinoma [30]. In a recent study, Yan et al. [31], described the diagnostic and prognostic value of $GABA_A$ receptors in patients with COAD. However, to the best of our knowledge, the potential roles of GABRD in the prognostic evaluation of patients with COAD have not yet been thoroughly and systematically determined.

This is the first study to thoroughly evaluate the correlation between GABRD mRNA expression and clinicopathological characteristics and to analyze the prognostic value of GABRD mRNA expression in COAD based on the data obtained from The Cancer Genome Atlas (TCGA) database. Furthermore, the GABRD-related biological pathways involved in COAD were determined by gene set enrichment analysis (GSEA), which may offer further insights into the molecular mechanisms underlying COAD. The results of this study indicated that GABRD mRNA can be a promising prognostic biomarker and a molecular therapeutic target for COAD.

Materials and methods

Bioinformatics analysis based on TCGA database

The GABRD mRNA expression data (Workflow Type: HTSeq-FPKM) and the corresponding clinical information of patients with COAD were obtained from TCGA official website (https://portal.gdc.cancer.gov/). When the mRNA had duplicate data, the average mRNA expression was used. In total, the data of 452 COAD tissues and 41 adjacent normal tissue data were obtained. For further analysis, 447 COAD cases were selected. The cases that did not include the clinical prognostic information and those with overall survival (OS) less than 30 days were excluded from further analysis. The clinical characteristics of patients, including age at diagnosis, gender, race, TNM stage, T stage, N stage, M stage, histologic type, primary tumor location, MSI status, and carcinoembryonic antigen (CEA) level before treatment were recorded. Unavailable or unknown clinical characteristics in 447 cases were regarded as missing values.

GSEA

GSEA is a computational approach to determine significantly enriched or depleted groups of genes [32]. In this study, we performed GSEA to explore potential molecular mechanisms underlying the effect of GABRD expression on COAD prognosis. The COAD samples were divided into GABRD mRNA high expression and low expression groups based on the median value of GABRD mRNA expression level. The "c2.cp.kegg.v7.0.symbols.gmt" gene sets from the Molecular Signatures Database (MSigDB) were analyzed using the GSEA 3.0 software. The normalized enrichment score (NES), nominal *p*-value, and false discovery rate (FDR) *q*-value were selected to classify the signaling pathways enriched in each phenotype. The number of gene set permutations for each analysis was set at 1000. The gene sets with p-value < 0.05 and FDR < 0.25 were regarded as significantly enriched.

Statistical analysis

Comparison of the GABRD mRNA expression in non-paired cases and paired cases were performed using the Wilcoxon rank-sum test and Wilcoxon matched-pairs signed-rank test, respectively. The median value of the GABRD mRNA expression was defined as the cut-off value. The correlation between GABRD mRNA expression and clinicopathologic characteristics was analyzed by Wilcoxon rank-sum test or Kruskal-Wallis test and logistic regression. The Kaplan-Meier method was used to generate the survival curve. The log-rank test was performed to compare the differences in OS. The univariate and multivariate Cox proportional hazard regression models were utilized to determine the effects of GABRD mRNA expression and clinicopathologic characteristics on OS. Moreover, the Harrell's concordance index (C-index) was used to evaluate the discrimination power of the model. All statistical analyses were performed using R 3.6.1 software (R Core Team, 2019). The difference was considered statistically significant when the *p*-value was less than 0.05.

Results

Patient characteristics

The data, including GABRD mRNA expression and clinical data, of 447 primary COAD cases were downloaded from TCGA database in Nov 2019. Of the 447 cases, 212 (47.4%) were female and 235 (52.6%) were male. The study cohort included 209 (75.2%) Caucasian and 69 (24.8%) non-Caucasian patients with median age of 69 years (range, 31–90 years). The TNM stages I, II, III, and IV accounted for 17.2%, 40.4%, 28.4% and 14.0% of the cases, respectively. The MSI status of COAD was microsatellite stable (MSS), MSI-low (MSI-L), and MSI-high (MSI-H) in 63.4%, 18.1% and 18.5% of the cases, respectively. Of the 391 cases, 61 (15.6%) cases had distant metastasis. Of the 429 cases with primary tumor location, 173 (40.3%) cases located toward the left of the colon. The histologic types of 86.2% ($n = 381$) and 13.8% ($n = 61$) of the tumors were adenocarcinoma and mucinous adenocarcinoma, respectively. The CEA level before treatment was less than 5 ng/mL in 66.1% of the cases ($n = 187$). The median survival time of COAD was 7.73 years, and the number of death was 72 (Table 1).

COAD tissues exhibit enhanced GABRD mRNA expression

The GABRD mRNA expression levels in the COAD tissues and normal tissues were compared using the Wilcoxon rank-sum test. The COAD tissues exhibited significantly higher GABRD mRNA expression levels than the normal tissues ($p < 0.0001$) (Fig. 1a). Additionally, the GABRD mRNA expression levels were analyzed in 41 paired COAD and adjacent non-

Table 1
Clinical characteristics of patients with colon adenocarcinoma based on the TCGA database.

Clinical characteristics		Total (n = 447)	Percentage (%)
Age at diagnosis (years)	Mean (SD)	67.1 (13.0)	
	Median [Min-Max]	69 [31–90]	
GABRD expression	Median [Min-Max]	0.693 [0.027–3.931]	
Gender	Female	212	47.4%
	Male	235	52.6%
Race	Non-Caucasian	69	24.8%
	Caucasian	209	75.2%
TNM stage	Stage I	75	17.2%
	Stage II	176	40.4%
	Stage III	124	28.4%
	Stage IV	61	14.0%
T stage	T1	10	2.2%
	T2	76	17.0%
	T3	304	68.2%
	T4	56	12.6%
N stage	N0	266	59.5%
	N1	102	22.8%
	N2	79	17.7%
M stage	M0	330	84.4%
	M1	61	15.6%
Histologic type	Adenocarcinoma	381	86.2%
	Mucinous adenocarcinoma	61	13.8%
Primary tumor location	Left	173	40.3%
	Right	256	59.7%
MSI status	MSS	267	63.4%
	MSI-L	76	18.1%
	MSI-H	78	18.5%
CEA level before treatment	<5 ng/mL	187	66.1%
	≥5 ng/mL	96	33.9%
Median survival time	Total	7.73	
Survival status	Death	72	18.4%
	Alive	319	83.6%

TCGA, The Cancer Genome Atlas; T, tumor; N, node; M, metastasis; MSI, microsatellite instability; MSS, microsatellite stable; MSI-L, MSI-low; MSI-H, MSI-high; CEA, carcinoembryonic antigen; SD, standard deviation.

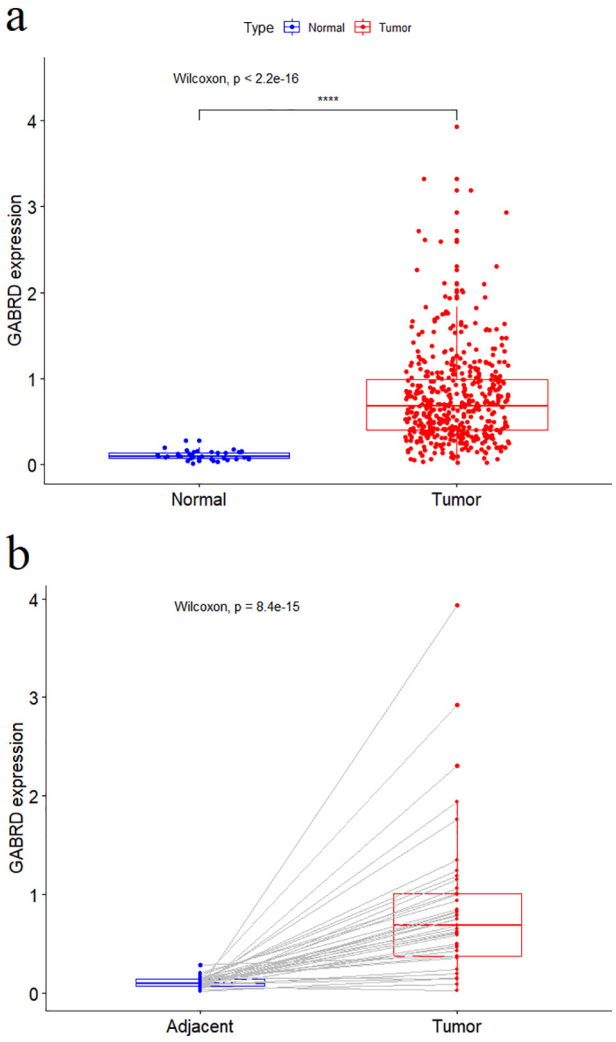

Fig. 1. The COAD tissues exhibited higher mRNA expression levels of GABRD than the normal or adjacent normal tissues. (**a**) GABRD mRNA expression in the tumor and normal tissues. (**b**) GABRD expression in 41 pairs of tumor and adjacent normal tissues. ****$p < 0.0001$. COAD, colon adenocarcinoma; GABRD, γ-aminobutyric acid type A receptor δ subunit.

tumorous tissues using Wilcoxon matched-pairs signed-rank test. The analysis revealed that GABRD mRNA was overexpressed in the COAD tissues ($p < 0.0001$) (Fig. 1b).

Correlation between GABRD mRNA expression and clinicopathologic characteristics in patients with COAD

The correlation between GABRD mRNA expression in 447 COAD samples and clinicopathologic characteristics of the corresponding patients was analyzed by Wilcoxon rank-sum test or Kruskal-Wallis test. As shown in Fig. 2a–f, the enhanced GABRD mRNA expression was significantly associated with TNM stage ($p = 0.00052$), T stage ($p = 0.012$), N stage ($p = 0.00031$), M stage ($p = 0.00043$), primary tumor location ($p = 0.028$), and MSI status ($p = 0.0069$). However, there was no significant correlation between the enhanced GABRD mRNA expression and gender, race, histologic type and CEA level, respectively. The COAD samples were divided into GABRD mRNA high and low expression groups based on the median GABRD expression level. The logistic regression analysis indicated that increased GABRD mRNA expression in COAD was observably correlated with TNM stage (odds ratio [OR] = 1.60 for stage III/IV vs. stage I/II, $p = 0.016$), N stage (OR = 1.67 for N1/N2 vs. N0, $p = 0.008$), M stage (OR = 2.15 for M1 vs. M0, $p = 0.009$), and MSI status (OR = 0.60 for MSI-H vs. MSS/MSI-L, $p = 0.049$) (Table 2). These results revealed that COAD with increased GABRD mRNA expression was prone to progress to the advanced TNM stage, lymph node stage, and distant metastasis and GABRD expression might have prognostic significance for COAD patients.

Survival outcomes and Cox regression analysis

To determine the prognostic value of GABRD mRNA expression in COAD, patients who lacked complete clinical information were excluded from the analysis. The Kaplan-Meier survival curve and log-rank test revealed that patients with COAD exhibiting high GABRD mRNA expression had a worse prognosis than patients with COAD exhibiting low GABRD mRNA expression ($p = 0.0062$) (Fig. 3). Additionally, we considered the importance of stage II tumors as there is a controversy on treating patients with stage II tumors by chemotherapy. There was no difference in the GABRD mRNA expression level between stage IIA and stage IIB tumors. Additionally, there was no difference in the survival of patients with different stage II subgroups. The univariate Cox regression analysis revealed that high GABRD mRNA expression was associated with poor OS (hazard ratio [HR] = 2.33, 95% confidence interval [CI] [1.40–3.88], $p = 0.001$; Table 3). Other clinicopathologic characteristics, such as age, TNM stage, T stage, N stage, and M stage were also significantly associated with OS (all p-values <0.05; Table 3). Moreover, multivariate analysis using the Cox proportional hazards model was performed to confirm the prognostic value of GABRD mRNA expression. Markers that were significant in univariate analyses were forward into the multivariate analysis and the three

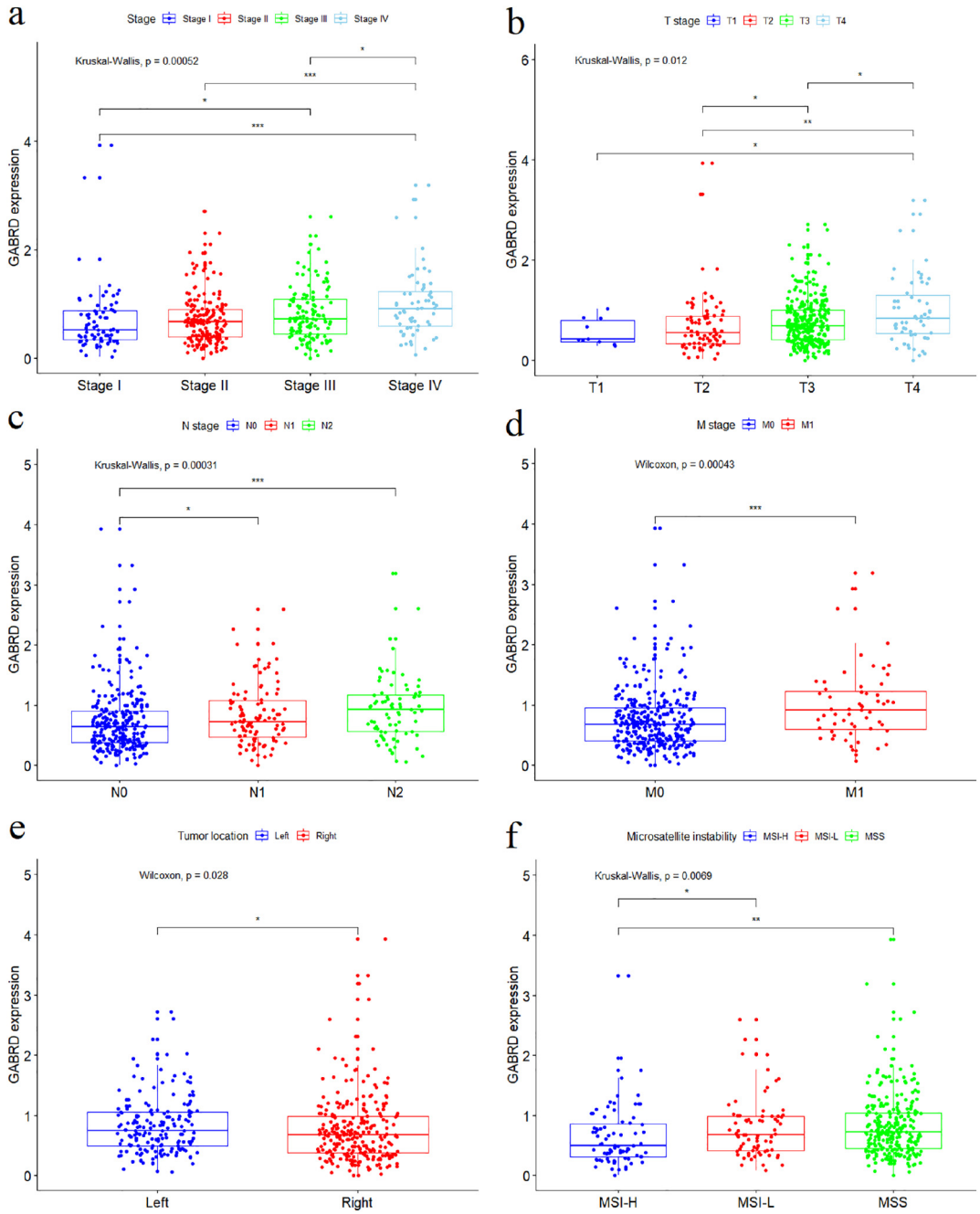

Fig. 2. Correlation between GABRD mRNA expression and clinicopathologic characteristics. (**a**) TNM stage, (**b**) T stage, (**c**) N stage, (**d**) M stage, (**e**) Primary tumor location, (**f**) MSI status. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. T, tumor; N, node; M, metastasis; GABRD, γ-aminobutyric acid type A receptor δ subunit; MSI, microsatellite instability; MSS, microsatellite stable; MSI-L, MSI-low; MSI-H, MSI-high.

markers: gender, histologic type, and microsatellite instability status were excluded. Finally, seven variables were included in the multivariate Cox analysis. We calculated the power for the multivariate analysis with all the markers and achieved 87% power using PASS software. The analysis revealed that high GABRD mRNA expression (HR = 2.14, 95% CI [1.26–3.64], $p = 0.005$), age (HR = 1.04, 95% CI [1.02–1.07], $p < 0.0001$), TNM stage (HR = 7.29, 95% CI [1.74–30.53], $p = 0.007$) and M stage (HR = 2.52, 95% CI [1.34–4.75], $p = 0.004$) were independently associated with OS (Table 3). The C-index of the model was 0.789 (95% CI 0.738–0.841) (se = 0.026), supporting the model suitability to predict the survival rate for COAD patients. These results indicated that the GABRD mRNA was an independent prognostic factor and increased GABRD mRNA level was associated with poor OS.

GABRD-related signaling pathways based on GSEA

GSEA was performed to identify the potential signaling pathways involved in COAD between low and high GABRD mRNA expression datasets. There were significant differences (FDR < 0.25, nominal p-value <0.05) in the enrichment of "c2.cp.kegg.v7.0.symbols.gmt" gene sets from the MSigDB between the low and high GABRD mRNA expression datasets. The GSEA revealed that GABRD was involved in signaling pathways including cell adhesion molecules (cams), gap

Table 2
Correlation of GABRD mRNA expressiona and clinicopathological characteristics (logistic regression analysis).

Clinical characteristics	Total (N)	Odds ratio for GABRD expression[a]	*p*-Value
Age (continuous)	447	0.99 (0.98–1.01)	0.397
Gender (female vs. male)	447	0.69 (0.48–1.01)	0.053
TNM Stage (stage III/IV vs. stage I/II)	436	1.60 (1.09–2.35)	0.016*
T stage (T3/T4 vs. T1/T2)	446	1.59 (0.99–2.58)	0.056
N stage (N1/N2 vs. N0)	447	1.67 (1.14–2.45)	0.008**
M stage (M1 vs. M0)	391	2.15 (1.23–3.86)	0.009**
Adenocarcinoma vs. Mucinous adenocarcinoma	442	1.41 (0.82–2.45)	0.216
Primary tumor location (right vs. left)	429	0.74 (0.50–1.09)	0.130
MSI-H vs. MSS/MSI-L	421	0.60 (0.36–0.99)	0.049*
CEA level before treatment (≥5 vs. <5)	283	1.15 (0.70–1.88)	0.586

GABRD, γ-aminobutyric acid type A receptor δ subunit; T, tumor; N, node; M, metastasis; MSI, microsatellite instability; MSS, microsatellite stable; MSI-L, MSI-low; MSI-H, MSI-high; CEA, carcinoembryonic antigen.
* $p < 0.05$.
** $p < 0.01$.
[a] Categorical dependent variable, greater or less than the median expression level.

junction, melanogenesis, and mTOR signaling pathway, as well as the signaling pathways associated with basal cell carcinoma or bladder cancer development (Table 4, Fig. 4).

Discussion

Colon cancer, a heterogeneous disease, is one of the leading causes of cancer-related deaths worldwide. The identification of prognostic factors and underlying molecular mechanisms of COAD can aid in the development of a novel therapeutic strategy [33]. Neurotransmitters in the tumor microenvironment can stimulate colon cancer cell growth and metastasis through binding to the corresponding neurotransmitter receptors [16]. However, there is limited knowledge of the role of GABRD in patients with COAD. In the present study, we reported that the COAD tissues exhibit upregulated GABRD mRNA levels when compared with the normal or adjacent normal tissues. The enhanced GABRD mRNA expression level in the COAD tissue was markedly correlated with TNM stage, N stage, M stage, and MSI status. Additionally, patients with COAD exhibiting high GABRD mRNA expression were associated with poor OS. Furthermore, the GABRD mRNA expression level was an independent prognostic factor in COAD. To further elucidate the signaling pathways associated with GABRD, GSEA was performed using the high and low GABRD expression datasets. The signaling pathways, including cell adhesion molecules (cams), gap junction, melanogenesis, and mTOR signaling pathway, as well as the signaling pathways associated with basal cell carcinoma or bladder cancer development were significantly enriched in the GABRD high expression group. It is necessary to determine whether GABRD can promote the tumor progression of the colon using a cellular and nude mouse model in our further work. Besides, it is indispensable to elucidate the underlying molecular mechanism between GABRD and the signaling pathway in colon cancer. These results indicate that GABRD may serve as a potential prognostic and therapeutic target for patients with COAD.

Our results are in line with the finding of Yan et al. [31], who demonstrated the high expression of GABRD were associated with poor prognosis of patients with COAD and could be used as a prognostic biomarker. However, their research focused on the relationship between all $GABA_A$ receptor genes and the prognosis of COAD, and the clinical data were slightly inadequate. When analyzing the correlation between GABRD and clinicopathological characteristics, only three variables of age, gender and TNM stage were included. In our study, the univariate Cox regression analysis revealed that clinicopathologic characteristics, such as age, T stage, N stage and M stage were also remarkably associated with OS. We demonstrated that age and M stage were independently associated with OS in the multivariate Cox proportional hazard regression model.

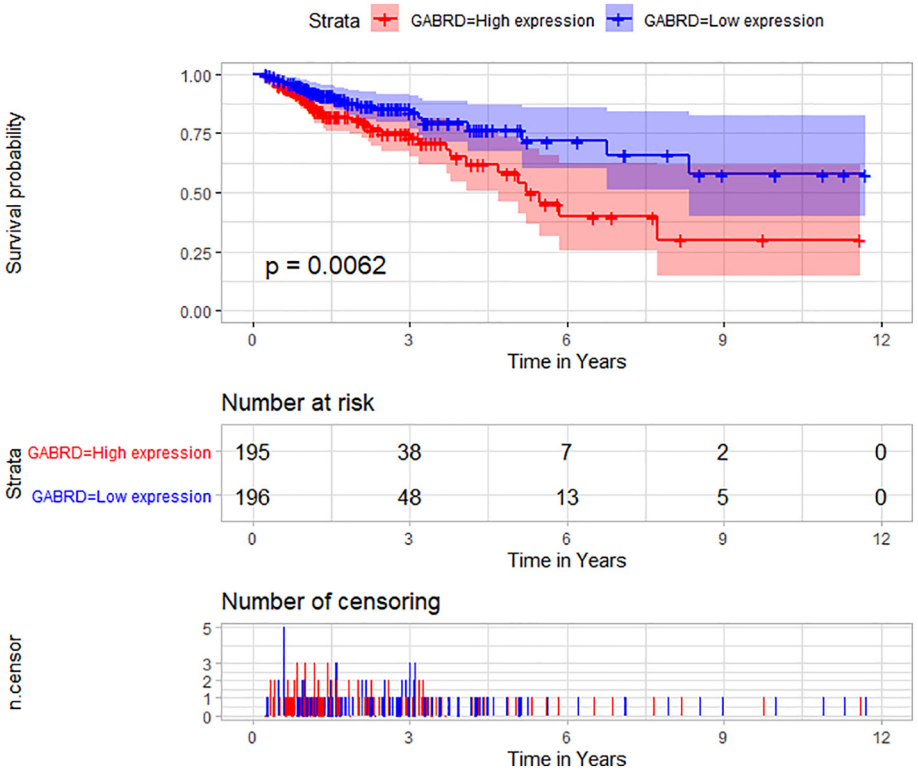

Fig. 3. High mRNA expression level of GABRD predicts poor overall survival in patients with COAD ($p = 0.0062$). The median value of GABRD mRNA expression was regarded as the cut-off value. COAD, colon adenocarcinoma; GABRD, γ-aminobutyric acid type A receptor δ subunit.

Table 3
Univariate and multivariate Cox proportional hazards analyses of overall survival in patients with colon adenocarcinoma.

Clinical characteristics	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Age (continuous)	1.03 (1.01–1.06)	0.004**	1.04 (1.02–1.07)	0.000***
Gender (female vs. male)	1.10 (0.67–1.80)	0.703		
TNM Stage (stage III/IV vs. stage I/II)	3.19 (1.90–5.34)	0.000***	7.29 (1.74–30.53)	0.007**
T stage (T3/T4 vs. T1/T2)	4.32 (1.35–13.81)	0.014*	1.91 (0.57–6.40)	0.294
N stage (N1/N2 vs. N0)	2.70 (1.63–4.45)	0.000***	0.30 (0.08–1.05)	0.060
M stage (M1 vs. M0)	4.53 (2.72–7.53)	0.000***	2.52 (1.34–4.75)	0.004**
Adenocarcinoma vs. Mucinous adenocarcinoma	1.69 (0.88–3.24)	0.114		
Primary tumor location (right vs. left)	1.64 (0.99–2.74)	0.055	1.59 (0.95–2.67)	0.079
Microsatellite instability (MSI-H vs. MSS/MSI-L)	1.07 (0.56–2.05)	0.842		
GABRD expression (high vs. low)	2.33 (1.40–3.88)	0.001**	2.14 (1.26–3.64)	0.005**

T, tumor; N, node; M, metastasis; HR, hazard ratio; CI, confidence interval; MSI, microsatellite instability; MSS, microsatellite stable; MSI-L, MSI-low; MSI-H, MSI-high; GABRD, γ-aminobutyric acid type A receptor δ subunit.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Previous studies have reported that several neurotransmitter receptors, which contribute to the malignant phenotype of colon cancer, are differentially expressed in the malignant colonic cells [34]. For example, the expression of neurokinin-1 (NK-1), a substance P (SP) receptor, is upregulated in COAD. Treatment with NK-1 antagonist can delay cell growth and induce cell death via apoptosis [35]. Another study by Ataee et al. has demonstrated that the HT29 colon cancer cells exhibit upregulated expression of 5-HT_{1B} serotonin receptor and that 5-HT_{1B} serotonin receptor antagonist exerts anti-proliferative and apoptotic effects on the HT29 cells [36]. In current study, bioinformatics analysis was employed to determine the prognostic value of GABRD mRNA expression in COAD using RNA sequencing data obtained from TCGA. To the best of our knowledge, this is the first study to identify GABRD mRNA expression as a prognostic factor for patients with COAD. In most cases, GABA stimulates cancer cell proliferation and metastasis through the $GABA_A$ receptor pathway. The gastric cancer tissues exhibit enhanced levels of GABA. Treatment with muscimol, a $GABA_A$ receptor agonist, can facilitate gastric cancer cell proliferation via the activation of mitogen-activated protein kinases (MAPKs) [27]. Gumireddy et al. demonstrated that the expression of GABRA3, a $GABA_A$ receptor subunit, is upregulated in human metastatic breast cancer, which was correlated to poor patient survival [25]. Similarly, Takehara et al. reported that GABA upregulates intracellular Ca^{2+} levels, activates the MAPK/ERK cascade, and stimulates pancreatic cancer growth by promoting overexpression of GABRP, a subunit of $GABA_A$ receptor [26]. In contrast, Jiang et al. reported that GABRP exhibits an immunomodulatory role in pancreatic cancer progression through GABA-independent tuning of KCNN4-mediated Ca^{2+} signaling [37].

The *GABRD* gene encodes the δ subunit of the $GABA_A$ receptor which is highly expressed in the brain and mediates signaling related to tonic inhibition [38]. The subunit expression is required for synaptic plasticity and neurogenesis [39,40]. The dysregulation of GABRD and single nucleotide polymorphisms (SNPs) in the *GABRD* gene are associated with childhood-onset mood disorder and generalized epilepsy [41,42]. Recently, Zhang et al. revealed that isocitrate dehydrogenase (IDH) wild-type tumors exhibit markedly lower GABRD expression than the IDH mutant diffuse low-grade glioma [29]. In addition, Sarathi et al. demonstrated that GABRD was significantly monotonically upregulated across TNM stage in hepatocellular carcinoma [30]. However, the role of GABRD in COAD has not been previously elucidated. In this study, elevated GABRD expression in COAD was associated with advanced clinicopathological features (TNM stage, T stage, N stage, M stage, and MSI status), which suggested that GABRD may play a critical role in COAD invasion and metastasis. Recurrence and distal metastases are the two main causes of cancer-related deaths. This explains the reason for the correlation of high GABRD mRNA expression with poor prognosis in COAD. However, further studies are required to investigate the metastasis mechanisms of GABRD in COAD, which may provide a novel therapeutic approach for COAD.

Although this study determined the prognostic value of GABRD in COAD, there are still some limitations. This study only used COAD data from TCGA and no validation dataset was used. Additionally, the conclusion of the study is limited to GABRD mRNA expression. Further studies are needed to evaluate GABRD protein expression and direct mechanisms. Furthermore, GABRD extracellular concentration is similar to hormonal

Table 4
Gene sets enriched in the colon adenocarcinoma tissues exhibiting high GABRD expression.

MSigDB collection	Gene set name	NES	NOM *p*-val	FDR *q*-val
c2.cp.kegg. v7.0. symbols.gmt	KEGG_NOTCH_SIGNALING_PATHWAY	1.981	0.006	0.281
	KEGG_BASAL_CELL_CARCINOMA	1.891	0.008	0.209
	KEGG_CELL_ADHESION_MOLECULES_CAMS	1.794	0.036	0.237
	KEGG_GAP_JUNCTION	1.737	0.023	0.238
	KEGG_ECM_RECEPTOR_INTERACTION	1.736	0.063	0.214
	KEGG_MELANOGENESIS	1.708	0.021	0.234
	KEGG_BLADDER_CANCER	1.682	0.022	0.234
	KEGG_FOCAL_ADHESION	1.674	0.063	0.227
	KEGG_MTOR_SIGNALING_PATHWAY	1.667	0.010	0.221
	KEGG_AXON_GUIDANCE	1.631	0.043	0.259

Gene set with NOM *p*-value < 0.05 and FDR *q*-value < 0.25 were regarded as significantly enriched. GABRD, γ-aminobutyric acid type A receptor δ subunit; NES, normalized enrichment score; NOM *p*-val, normalized *p*-value; FDR, false discovery rate.

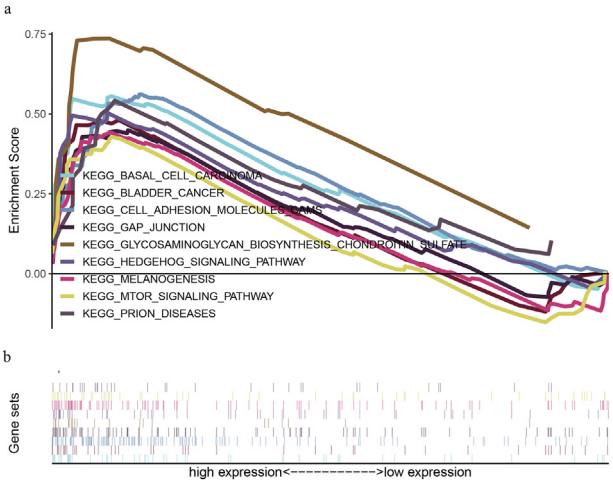

Fig. 4. Enrichment plots from GSEA. (**a**) Enrichment score and (**b**) gene sets. GSEA, gene set enrichment analysis.

concentration. Therefore, further methodological adjustments are needed to increase the sensitivity of the detection and quantification of GABRD.

Conclusions

This study demonstrated that the COAD tissues exhibited GABRD mRNA overexpression and that GABRD mRNA expression may be a potential prognostic marker for patients with COAD. Further studies are needed to elucidate the molecular mechanism underlying the role of GABRD in the tumor microenvironment in facilitating cancer invasion and metastasis.

CRediT authorship contribution statement

Moxin Wu and Moon Young Lee conceived and designed the study and prepared the manuscript. Keun Young Kim and Won Cheol Park acquired, collected, and extracted the data included in this analysis. Han-Seung Ryu, Suck Chei Choi and Min Seob Kim analyzed the data. Ji Yeon Myung, Hyun Seok Choi and Eui Joong Kim helped with manuscript preparation and data review. All authors have read and approved the final version manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, Jemal, A. Global cancer statistics, GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J. Clin. 2018 (68) (2018) 394–424.
[2] M. Fleming, S. Ravula, S.F. Tatishchev, H.L. Wang, Colorectal carcinoma: pathologic aspects, J. Gastrointest. Oncol. 3 (2012) 153–173.
[3] Z. Zhai, X. Yu, B. Yang, Y. Zhang, L. Zhang, X. Li, H. Sun, Colorectal cancer heterogeneity and targeted therapy: clinical implications, challenges and solutions for treatment resistance, Semin. Cell Dev. Biol. 64 (2017) 107–115.
[4] X. Sagaert, Prognostic biomarkers in colorectal cancer: where do we stand? Virchows Arch. 464 (2014) 379–391.
[5] I.D. Nagtegaal, P. Quirke, H.J. Schmoll, Has the new TNM classification for colorectal cancer improved care? Nat. Rev. Clin. Oncol. 9 (2011) 119–123.
[6] A.R. Sepulveda, S.R. Hamilton, C.J. Allegra, W. Grody, A.M. Cushman-Vokoun, W.K. Funkhouser, S.E. Kopetz, C. Lieu, N.M. Lindor, B.D. Minsky, et al., Molecular biomarkers for the evaluation of colorectal cancer: guideline from the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and the American Society of Clinical Oncology, J. Clin. Oncol. 35 (2017) 1453–1486.
[7] E. Koncina, S. Haan, S. Rauh, E. Letellier, Prognostic and predictive molecular biomarkers for colorectal cancer: updates and challenges, Cancers (Basel) 12 (2020) 319.
[8] F. Pagès, B. Mlecnik, F. Marliot, G. Bindea, F.S. Ou, C. Bifulco, A. Lugli, I. Zlobec, T.T. Rau, M.D. Berger, et al., International validation of the consensus immunoscore for the classification of colon cancer: a prognostic and accuracy study, Lancet 391 (2018) 2128–2139.
[9] A.C. Rogers, D.C. Winter, A. Heeney, D. Gibbons, A. Lugli, G. Puppa, K. Sheahan, Systematic review and meta-analysis of the impact of tumour budding in colorectal cancer, Br. J. Cancer 115 (2016) 831–840.
[10] F.A. Sinicrope, M.R. Mahoney, T.C. Smyrk, S.N. Thibodeau, R.S. Warren, M.M. Bertagnolli, G.D. Nelson, R.M. Goldberg, D.J. Sargent, S.R. Alberts, Prognostic impact of deficient DNA mismatch repair in patients with stage III colon cancer from a randomized trial of FOLFOX-based adjuvant chemotherapy, J. Clin. Oncol. 31 (2013) 3664–3672.
[11] G. Lorusso, C. Ruegg, The tumor microenvironment and its contribution to tumor evolution toward metastasis, Histochem. Cell Biol. 130 (2008) 1091–1103.
[12] M. Quante, J. Varga, T.C. Wang, F.R. Greten, The gastrointestinal tumor microenvironment, Gastroenterology 145 (2013) 63–78.
[13] P. Jobling, J. Pundavela, S.M. Oliveira, S. Roselli, M.M. Walker, H. Hondermarck, Nerve-cancer cell cross-talk: a novel promoter of tumor progression, Cancer Res. 75 (2015) 1777–1781.
[14] D. Albo, C.L. Akay, C.L. Marshall, J.A. Wilks, G. Verstovsek, H. Liu, N. Agarwal, D.H. Berger, G.E. Ayala, Neurogenesis in colorectal cancer is a marker of aggressive tumor behavior and poor outcomes, Cancer 117 (2011) 4834–4845.
[15] M. Amit, S. Na'ara, Z. Gil, Mechanisms of cancer dissemination along nerves, Nat. Rev. Cancer 16 (2016) 399–408.
[16] N. Kuol, L. Stojanovska, V. Apostolopoulos, K. Nurgali, Role of the nervous system in cancer metastasis, J. Exp. Clin. Cancer Res. 37 (2018) 5.
[17] C. Sarkar, D. Chakroborty, S. Basu, Neurotransmitters as regulators of tumor angiogenesis and immunity: the role of catecholamines, J. NeuroImmune Pharmacol. 8 (2013) 7–14.
[18] K. Kanbara, Y. Otsuki, M. Watanabe, S. Yokoe, Y. Mori, M. Asahi, M. Neo, $GABA_B$ receptor regulates proliferation in the high-grade chondrosarcoma cell line OUMS-27 via apoptotic pathways, BMC Cancer 18 (2018) 263.
[19] S.H. Jiang, L.P. Hu, X. Wang, J. Li, Z.G. Zhang, Neurotransmitters: emerging targets in cancer, Oncogene 39 (2020) 503–515.
[20] X. Zhang, Z. Du, J. Liu, J. He, Gamma-aminobutyric acid receptors affect the progression and migration of tumor cells, J. Recept. Signal Transduct. Res. 34 (2014) 431–439.
[21] J. Joseph, B. Niggemann, K.S. Zaenker, F. Entschladen, The neurotransmitter γ-aminobutyric acid is an inhibitory regulator for the migration of SW 480 colon carcinoma cells, Cancer Res. 62 (2002) 6467–6469.
[22] M. Vithlani, M. Terunuma, S.J. Moss, The dynamic modulation of GABA(A) receptor trafficking and its role in regulating the plasticity of inhibitory synapses, Physiol. Rev. 91 (2011) 1009–1022.
[23] M. Abdul, S.D. Mccray, N.M. Hoosein, Expression of gamma-aminobutyric acid receptor (subtype A) in prostate cancer, Acta Oncol. 47 (2008) 1546–1550.
[24] Y.H. Li, Y. Liu, Y.D. Li, Y.H. Liu, F. Li, Q. Ju, P.L. Xie, G.C. Li, GABA stimulates human hepatocellular carcinoma growth through overexpressed GABAA receptor theta subunit, World J. Gastroenterol. 18 (2012) 2704–2711.
[25] K. Gumireddy, A. Li, A.V. Kossenkov, M. Sakurai, J. Yan, Y. Li, H. Xu, J. Wang, P.J. Zhang, L. Zhang, The mRNA-edited form of GABRA3 suppresses GABRA3-mediated Akt activation and breast cancer metastasis, Nat. Commun. 7 (2016) 1–9.
[26] A. Takehara, M. Hosokawa, H. Eguchi, H. Ohigashi, O. Ishikawa, Y. Nakamura, H. Nakagawa, Gamma-aminobutyric acid (GABA) stimulates pancreatic cancer growth through overexpressing GABAA receptor pi subunit, Cancer Res. 67 (2007) 9704–9712.
[27] K. Maemura, N. Shiraishi, K. Sakagami, K. Kawakami, T. Inoue, M. Murano, M. Watanabe, Y. Otsuki, Proliferative effects of gamma-aminobutyric acid on the gastric cancer cell line are associated with extracellular signal-regulated kinase 1/2 activation, J. Gastroenterol. Hepatol. 24 (2009) 688–696.
[28] Z. Kleinrok, M. Matuszek, J. Jesipowicz, B. Matuszek, A. Opolski, C. Radzikowski, GABA content and GAD activity in colon tumors taken from patients with colon cancer or from xenografted human colon cancer cells growing as s.c. tumors in athymic nu/nu mice, J. Physiol. Pharmacol. 49 (1998) 303–310.
[29] H. Zhang, L. Zhang, Y. Tang, C. Wang, Y. Chen, J. Shu, K. Zhang, Systemic screening identifies GABRD, a subunit gene of GABAA receptor as a prognostic marker in adult IDH wild-type diffuse low-grade glioma, Biomed. Pharmacother. 118 (2019) 109215.
[30] A. Sarathi, A. Palaniappan, Novel significant stage-specific differentially expressed genes in hepatocellular carcinoma, BMC Cancer 19 (2019) 663.
[31] L. Yan, Y.Z. Gong, M.N. Shao, G.T. Ruan, H.L. Xie, X.W. Liao, X.K. Wang, Q.F. Han, X. Zhou, L.C. Zhu, et al., Distinct diagnostic and prognostic values of γ-aminobutyric acid type A receptor family genes in patients with colon adenocarcinoma, Oncol. Lett. 20 (2020) 275–291.
[32] A. Subramanian, P. Tamayo, V.K. Mootha, S. Mukherjee, B.L. Ebert, M.A. Gillette, A. Paulovich, S.L. Pomeroy, T.R. Golub, E.S. Lander, et al., Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 15545–15550.
[33] L. Duan, W. Yang, X. Wang, W. Zhou, Y. Zhang, J. Liu, H. Zhang, Q. Zhao, L. Hong, D. Fan, Advances in prognostic markers for colorectal cancer, Expert. Rev. Mol. Diagn. 19 (2019) 313–324.
[34] G. Rademakers, N. Vaes, S. Schonkeren, A. Koch, K.A. Sharkey, V. Melotte, The role of enteric neurons in the development and progression of colorectal cancer, Biochim. Biophys. Acta. Rev. Cancer 2017 (1868) 420–434.
[35] M. Rosso, M.J. Robles-Frias, R. Covenas, M.V. Salinas-Martin, M. Munoz, The NK-1 receptor is expressed in human primary gastric and colon adenocarcinomas and is involved in the antitumor action of L-733,060 and the mitogenic action of substance P on human gastrointestinal cancer cell lines, Tumour Biol. 29 (2008) 245–254.
[36] R. Ataee, S. Ajdary, M. Zarrindast, M. Rezayat, M.R. Hayatbakhsh, Anti-mitogenic and apoptotic effects of 5-HT1B receptor antagonist on HT29 colorectal cancer cell line, J. Cancer Res. Clin. Oncol. 136 (2010) 1461–1469.
[37] S.H. Jiang, L.L. Zhu, M. Zhang, R.K. Li, Q. Yang, J.Y. Yan, C. Zhang, J.Y. Yang, F.Y. Dong, M. Dai, et al., GABRP regulates chemokine signalling, macrophage recruitment and tumour progression in pancreatic cancer through tuning KCNN4-mediated Ca (2+) signalling in a GABA-independent manner, Gut 2019 (1994-2006) 68.
[38] C. Windpassinger, P.M. Kroisel, K. Wagner, E. Petek, The human γ-aminobutyric acid A receptor delta (GABRD) gene: molecular characterisation and tissue-specific expression, Gene 292 (2002) 25–31.
[39] P.D. Whissell, S. Avramescu, D.S. Wang, B.A. Orser, δGABAA receptors are necessary for synaptic plasticity in the hippocampus: implications for memory behavior, Anesth. Analg. 123 (2016) 1247–1252.
[40] P.D. Whissell, S. Rosenzweig, I. Lecker, D.S. Wang, J.M. Wojtowicz, B.A. Orser, γ-aminobutyric acid type A receptors that contain the δ subunit promote memory and neurogenesis in the dentate gyrus. *Ann*, Neurol. 74 (2013) 611–621.
[41] L.M. Dibbens, H.J. Feng, M.C. Richards, L.A. Harkin, B.L. Hodgson, D. Scott, M. Jenkins, S. Petrou, G.R. Sutherland, I.E. Scheffer, GABRD encoding a protein for extra-or perisynaptic GABAA receptors is a susceptibility locus for generalized epilepsies, Hum. Mol.Genet. 13 (2004) 1315–1319.
[42] Y. Feng, K. Kapornai, E. Kiss, Z. Tamás, L. Mayer, I. Baji, G. Daróczi, I. Benák, V.O. Kothencné, E. Dombovári, Association of the GABRD gene and childhood-onset mood disorders, Genes Brain Behav. 9 (2010) 668–672.