Original Article

Linking the Defect of Gamma-Aminobutyric Acid Type A Receptor Subunit Delta Gene to the Microsatellite Instability in Colorectal Cancer

Afraa Jawad Mamoori, Haider A. Alkafaji

Department of Pathology and Forensic Medicine, College of Medicine, University of Babylon, Hillah, Iraq

Abstract

Introduction: There is primitive data suggest the role of gamma-aminobutyric acid type A receptor subunit delta (GABRD) in the pathogenesis of colorectal cancer. This research aimed to investigate the role of GABRD in patients with microsatellite instability (MSI)-associated colorectal cancer. **Materials and Methods:** In total, 58 patients diagnosed with MSI-associated adenocarcinoma of colorectal cancer participated in this study. The level of GABRD expression in both cancerous and noncancerous colonic and rectal tissue was done first by immunohistochemistry and then confirmed by quantitative real-time polymerase chain reaction (PCR). In addition, GABRD expression level was correlated with other pathologic parameters of patients. **Results:** Overall, 72.41% of cases expressed a high level of GABRD at the genetic level, and 67.24% expressed GABRD at the protein level. Moreover, the adjacent normal tissue did not show specific expression of GABRD protein by immunohistochemical analysis. Also, this study demonstrates a significant correlation between GABRD expression to the advanced stages of MSI-associated adenocarcinoma of colorectal cancer. **Conclusion:** GABRD can be a new and important diagnostic marker in patients with MSI and can be targeted for the treatment of patients with colorectal cancer having MSI. Further studies with larger sample sizes and in vitro studies will be of great value to uncover the pathway by which this gene controls the defect in mismatch repair (MMR) genes.

Keywords: Colorectal cancer, GABRD gene, MSI-associated adenocarcinoma

Introduction

Colorectal cancer represents one of the most common life-threatening cancers in Western countries, with a remarkable increase in incidence in developing countries in the last decades. Molecular abnormalities play an important role in the pathogenesis and response of this disease to medical treatment. Approximately 15% of colorectal cancer cases are linked to microsatellite instability (MSI).[1] Although most cases of MSI in colorectal cancer are sporadic, MSI forms the whole molecular alteration of the hereditary form of colorectal cancer, known as (hereditary nonpolyposis colorectal cancer).[2]

MSI is the deficiency or defect that occurs in MMR genes (mismatch repair genes), which can occur due to single nucleotide substitution or polymorphism in the short nucleotide sequence of these genes.[3] At the time that the germline mutation of MMR genes is responsible for the hereditary form of colorectal cancer, epigenetic inactivation of these genes is the cause of a sporadic type of MSI-related colorectal cancer.[3] Recently, data highlight the role of MSI in determining the role and efficacy of some immune-based treatments and chemotherapeutic treatments of colorectal cancer.

GABRD (gamma-aminobutyric acid type A receptor subunit delta) is an important inhibitory neurotransmitter of the nervous system, belonging to gamma-aminobutyric acid.[4] Neurotransmitters can initiate cancer by developing signaling pathways that affect tumor microenvironment.[5]

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Address for correspondence: Dr. Afraa Jawad Mamoori, Department of Pathology and Forensic Medicine, College of Medicine, University of Babylon, Hillah, Iraq. E-mail: afraajawad@yahoo.com

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In addition, it can promote cancer progression via its effects on immune cells, endothelial cells, and stromal cells.[6] Previously, this gene has been identified with a fundamental role in epilepsy of early-onset childhood and mood disorders.[7] Recently, there is emerging data revealed that GABRD plays an essential role in some types of tumors like Esophageal cancer and Glioma.[8,9] Studies regarding its role in colorectal cancer are still in the primitive stage; few studies show that GABRD is associated with poor prognosis and metastasis of this disease.[10,11] This study was designed to determine the association of GABRD expression with MSI in patients with colorectal cancer.

Materials and Methods

Tissue samples

Formalin-fixed paraffin-embedded human tissue samples were prospectively collected from patients with colorectal cancer with corresponding noncancer tissues in private clinics in Iraq after obtaining consent from the patients. The study was approved by the ethical committee of scientific research at the University of Babylon in Iraq (Ref. No. 5-6/10/10/2022).

Clinical and pathologic data of the participating patients

After tissue collection, histopathological confirmation of a diagnosis of the collected samples from the surgical specimens was done. In total, 58 patients diagnosed with MSI-associated adenocarcinoma of colorectal cancer were selected for this study. Diagnosis of MSI was made by assessing the level of MLH1, MSH2, and MSH6 immunohistochemically. All of these cases are sporadic types of MSI. Staging of disease was done according to the 8th edition of the Cancer Staging Manual of the American Joint Committee on Cancer.[12] Grading of this disease was done according to the World Health Organization.[13] All clinical information of the selected patients was provided by tables shown in the *Results* section.

Immunohistochemical analysis

Immunohistochemical analysis is done according to our previously published protocol by using A high PH ENVisionTM FLEX Mini Kit (Dako, Glostrup, Hovedstaden, Denmark).[14] A polyclonal antibody against GABRD (1:100 dilutions; rabbit antihuman, mouse, and rat; abs141150; Absin Bioscience Inc., Shanghai, China) is used as the primary antibody. Colon cancer slide section, which is stain strongly for this antibody, is used as a positive control for each run. Another section was exposed to the same procedure, except that the section does not subjected to the primary antibody and was used as a negative control in each run. The slides after staining are classified to score 0 (the slide shows no staining for GABRD), score 1 (the slide shows 1%–30% staining for GABRD), score 2 (the slide shows 31% to 70% staining for GABRD), score 3 (the slide shows more than 70% staining for GABRD). All slides are assessed by two different pathologists.

Real-time PCR (quantitative type)

GABRD expression is quantified by using an IQ5 Multicolour Real-Time polymerase chain reaction (PCR) Detection system (Bio-Rad, Hercules, California) following our previous research.[14] quantitative real-time PCR was performed after total RNA extraction and cDNA conversion according to manufacturer guidelines (Qiagen miRNeasy Mini Kit [Qiagen, Germany]). The forward and reverse primers of GABRD are designed as the same sequence of nucleotides which is published before.[10] Primer specificity was detected by electrophoresis.

Statistical analysis

For analyzing data, the Statistical Package for Social Sciences (SPSS, version 22.0, IBM, New York, New York) was used. Regarding categorical variables, the Chi-square test was used. Pearson correlation test and independent *t*-test were used for continuous variables. A statistically significant result was achieved at *P* value of <0.05.

Ethical Approval

This study was approval by the ethical committee of scientific research at the University of Babylon in Iraq (Ref. No. 5-6 on 10/10/2022).

Results

Gamma-aminobutyric acid type A receptor subunit delta express in most cases of microsatellite instability of colorectal cancer

The result of quantitative real-time PCR demonstrates high expression of GABRD in patients suffering from adenocarcinoma who participated in this study (42 cases out of a total of 58 cases, 72.41%). Immunohistochemical analysis shows that this protein expresses in most cases, although its expression here is less than that of quantitative real-time PCR by 3 cases (39 cases out of a total of 58 cases, 67.24%). Our slides show nuclear staining, as shown in [Figures 1–3]. The adjacent normal tissues show negative or nonspecific staining of GABRD protein in all the cases which were included in this study [Figure 4].

Gamma-aminobutyric acid type A receptor subunit delta correlates significantly with the stage of colorectal cancer having microsatellite instability

Analysis of data shows that GABRD expresses more in advanced stages (III and IV). Both the gene and its protein are highly expressed in the advanced stages of this disease (*P* value < 0.05) [Tables 1 and 2]. The score of immunohistochemical staining is significantly higher in stage III and stage IV. Both studies revealed that there is no association between the expression of GABRD and other clinical and pathologic data of patients, which include the grade, gender, and age of the patients (*P* value >0.05) [Tables 2-5].

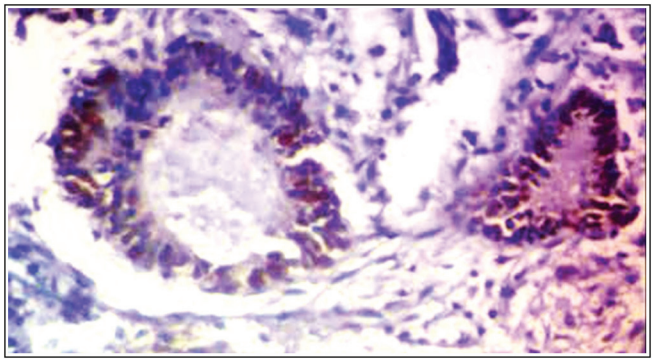

Figure 1: Well-differentiated adenocarcinoma (grade I) of MSI-associated colorectal cancer stain positive for GABRD (score 1)

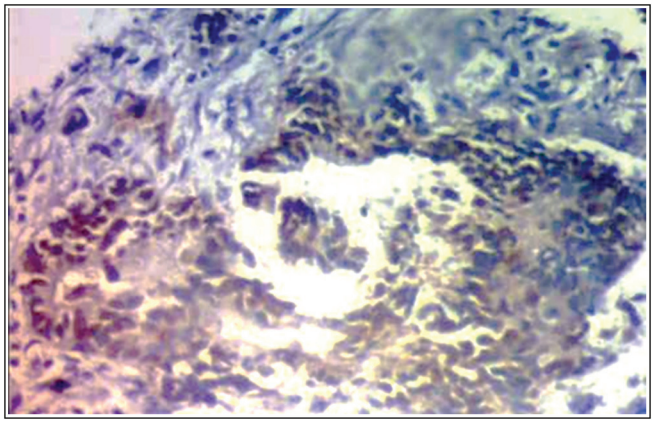

Figure 2: Moderately differentiated adenocarcinoma (grade II) of MSI-associated colorectal cancer stain positive for GABRD (score 2)

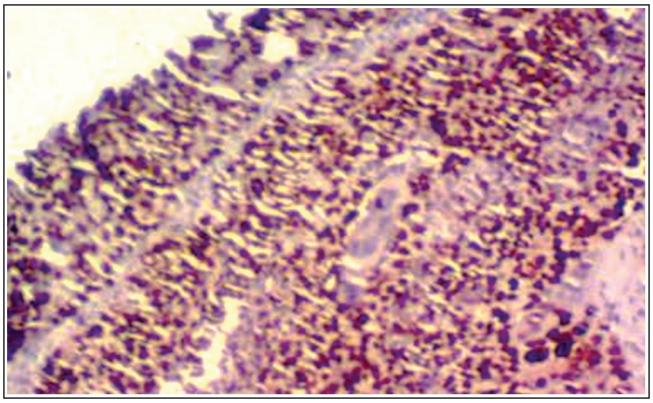

Figure 3: Poorly differentiated adenocarcinoma (grade III) of MSI-associated colorectal cancer stain positive for GABRD (score 3)

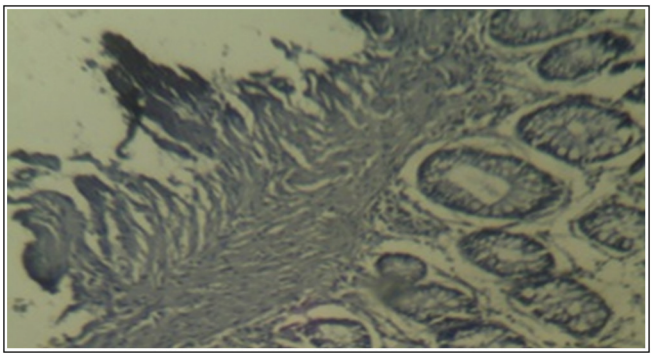

Figure 4: Normal colonic tissue stain negative with GABRD antibody

Table 1: Correlation of GABRD expression to the stage of disease by immunohistochemical study

Stage of disease	GABRD scoring				Total
	0	I	II	III	
I + II	11 (50%)	4 (18.18)	5 (22.72)	2 (9.09)	22 (27.93)
III + IV	8 (22.22)	0 (0%)	6 (16.66)	22 (61.11)	36 (62.06)
Total	19 (32.75)	4 (6.89)	11 (18.96)	24 (41.37)	58 (100%)

P value < 0.05

Table 2: GABRD expression (PCR study) in relation to clinical and pathologic parameters of patients

Parameters	Total number	High expression	Low or normal expression	*P* value
Age				
≤40	11 (18.96%)	8 (72.72%)	3 (27.27%)	0.71
>40	47 (81.03%)	34 (72.34%)	13 (27.65%)	
Gender				
Male	31 (53.44%)	23 (74.19%)	8 (25.80%)	0.62
Female	27 (46.55%)	19 (70.37%)	8 (29.62%)	
Grade				
I	16 (27.58%)	10 (62.5%)	6 (37.5%)	0.61
II	26 (44.82%)	19 (73.07%)	7 (26.92%)	
III	16 (27.58%)	13 (81.25%)	3 (18.75%)	
Stage				
I/II	22 (37.93%)	12 (54.54%)	8 (36.36%)	0.04
III/IV	36 (62.06%)	30 (83.33%)	8 (22.22%)	

Discussion

This study demonstrates the correlation of GABRD gene and its protein with MSI in colorectal cancer. To our knowledge, this is the first study designated to validate the role of GABRD in patients with colorectal cancer having MSI. A previous study showed that GABRD mRNA expression correlated significantly with MSI of colorectal cancer by using information from TCGA (The Cancer Genome Atlas) database and not validated in a set of patients.[11] No assessment for GABRD protein at the tissue level in patients with MSI in previously published data. Here is the significant elevation of mRNA GABRD expression in participating patients. Moreover, its protein expression shows a high level in most cases, although data did not reach a significant value. This may have been attributed to the difference in the condition associated with immunohistochemical analysis, which is used to measure the protein level and because quantitative real-time PCR, which is used to measure mRNA, is more sensitive procedure.

It's well known that MSI in sporadic colorectal cancer occurs due to epigenetic modification of DNA methylation of CpG islands in the promoter region of *MLH1* that

Table 3: Correlation of GABRD expression to the age of the patients by immunohistochemical study

Age-group	Immunostaining of GABRD		Total
	Positive	Negative	
Less than 40 years	7 (63.63)	4 (36.36)	11 (18.96)
More than 40 years	32 (68.08)	15 (31.91)	47 (81.03)
Total	39 (67.24)	19 (32.75)	58 (100%)

P value > 0.05

Table 4: Correlation of GABRD expression to the gender of the patients by immunohistochemical study

Gender	Immunostaining of GABRD		Total
	Positive	Negative	
Male	22 (64.70)	12 (35.29)	34 (58.62%)
Female	17 (70.83)	7 (29.16)	24 (41.37%)
Total	39 (67.24%)	19 (32.75%)	58 (100%)

P value > 0.05

Table 5: Correlation of GABRD expression to the grades of the tumor by immunohistochemical study

Grades of tumor	GABRD scoring				Total
	0	1	2	3	
Grade I	7 (43.75)	1 (6.25)	3 (18.75)	5 (62.5)	16 (31.25)
Grade II	8 (30.76)	5 (19.23)	2 (7.69)	11 (42.30)	26 (44.82)
Grade III	4 (25%)	5 (31.25)	4 (25%)	3 (18.75)	16 (27.58)
Total	19 (32.75)	11 (18.96)	9 (15.51)	19 (32.75)	58 (100%)

P value > 0.05

results in transcriptional silencing of this gene which is responsible for 80% of the cases.[15,16] There is no published data explaining the association of GABRD defect to the epigenetic silencing of *MLHI*. Furthermore, no previous linking of GABRD status to other MMR genes, which include *MSH2, MSH6*, and *PMS2*. This research opens the door to investigating the role of GABRD in MMR gene defects in colorectal cancer; the matter will add beneficial information that may result in targeting this gene and its protein in the treatment of patients with MSI. Moreover, it may reveal the causes behind the resistance of some patients to chemotherapy and immunotherapy, which are used as a treatment for this disease.

Our data show a significant correlation of GABRD expression with the stage of colorectal cancer (*P* value <0.05). This finding is consistent with previous research.[10,11] Recently, research reported that cell adhesion molecules (cams), gap junction, and mTOR signaling pathways are significantly enriched in patients having elevated GABRD gene expression in colorectal cancer adenocarcinoma.[17,18] This finding can be further investigated to uncover the direct and indirect roles of GABRD on the different genes involved in these signaling pathways. Further research with a larger sample size is required to support our findings.

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Conflicts of interest
There are no conflicts of interest.

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