



Gamma-Aminobutyric Acid Type A Receptor Subunit Pi is a potential chemoresistance regulator in colorectal cancer

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Abstract

Background Colorectal cancer (CRC) is one of the cancers with high morbidity and mortality worldwide. Chemotherapy is commonly used for metastatic or more advanced CRC. The mechanism of CRC chemoresistance is still under active investigation. Therefore, we identify and validate differentially expressed genes (DEGs) between oxaliplatin/5-FU resistant and sensitive CRC cells.

Methods and results Three datasets of colorectal cancer patients (GSE28691, GSE81006, and GSE77932) from the Gene Expression Omnibus (GEO) database were analyzed and volcano plots for DEGs were generated using the GEO2R tool. The intersection of three GEO datasets showed that GABRP was significantly upregulated in chemo-resistant CRC cells or patients with an adjusted p-value less than 0.01. The potential protein–protein interaction (PPI) network with GABRP was analyzed by the Search Tool for the Retrieval of Interaction Gene/Proteins (STRING) website. The PPI network predicted ANKRD66, CLINT1, HAP1, PLCL1, GABARPAP, GABARAPL1, NSF, GABARAPL2, TRAK2, and CLIC3 had a high likelihood to interact with GABRP. Especially, GABARAP, GABARAPL1, ANKRD66, CLINT1, and CLIC3 were enriched as the most possibly associated proteins with GABRP among the networks. GABRP was significantly more expressed in both oxaliplatin/5-FU resistant CRC cells than in those counterpart sensitive CRC cells using quantitative PCR (qPCR) analysis. Consistently, TCGA, Oncomine, and Human Protein Atlas (HPA) databases confirmed that higher expression of GABRP was robustly found in CRC patients than those in other various cancer types or normal colon tissues.

Conclusion We identify GABRP as a promising drug target to mediate oxaliplatin or 5-FU resistance in CRC. It provided the theoretical basis and potential clinical value for CRC patients.

Keywords GABRP · Colorectal cancer · Drug resistance · Oxaliplatin · 5-Fu · GEO

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Introduction

Colorectal cancer (CRC) is a common disease among the lethal cancer types worldwide with the second leading cause of cancer death among all cancers [1, 2]. Furthermore, the age-standardized incidence of CRC has increased by nearly 10% over the past two decades [3]. Although the prompt screening and related treatments have led to clinical improvement in CRC, the prognosis was relatively poor and 30% of patients developed tumor metastasis [4]. Clinically, chemotherapy generally includes oxaliplatin and 5-fluorouracil (5-Fu), which were commonly used as the first- or second-line therapy for metastatic colorectal cancer patients [5, 6].

Oxaliplatin, as one of the derivatives of cisplatin, additionally inhibits both DNA and protein synthesis and has a

relatively higher efficacy to suppress the cancer cell proliferation during the course of chemotherapy [7, 8]. Curative resection of CRC is often followed by adjuvant chemotherapy mainly based on oxaliplatin, for example, CAPOX (oxaliplatin and capecitabine) [9]. Immune checkpoint inhibitors, recommended as the pioneering treatment, have shown better therapeutic activity in microsatellite instability-high (MSI-H) or deficient mismatch repair (dMMR) CRC patients [10]. Recent studies have indicated that oxaliplatin can improve PD-1 therapy by combination with Trifluridine/Tipiracil in CRC [11]. Long-term oxaliplatin treatment may accumulate acquired chemoresistance, which the mechanism is still unknown.

Since the 1990s, fluorouracil had been the mainstay of colorectal cancer chemotherapy and 5-Fu remained one of the main constituents of current combinational chemotherapy in CRC [12]. As an analog of uracil, 5-Fu has a fluorine atom instead of hydrogen at the C5 position and inhibited tumor growth [13, 14]. Likewise, acquired drug resistance remained the bottleneck of CRC treatments. Our study generated both oxaliplatin and 5-Fu resistant CRC cells using elevated doses of both drugs to stimulate the cancer cells over a long period of time. We are devoted to identifying potential differentially expressed genes (DEGs) between sensitive and resistant CRC cell lines.

HCT-8/5-Fu and SW480/LOPH were two CRC-resistant cell lines. We screened DEGs from GEO data mining by comparing our cell line models of chemo-sensitive and corresponding chemo-resistant cells. Gamma-Aminobutyric Acid Type A Receptor Subunit Pi (GABRP) ranked as the top candidate gene. GABRP is the π subunit of the GABA_A receptor. γ -aminobutyric acid (GABA) is one of the most significant inhibitory neurotransmitters in the adult mammalian brain and has two categories of receptors: ionotropic (GABA_A or GABA_C) and metabotropic (GABA_B) receptors [15]. The GABA_A receptor has 19 subunits (α 1–6, β 1–3, γ 1–3, δ , ϵ , θ , π , and ρ 1–3) and GABRP is an oncogene in many cancers including gastroenteric tumors, such as gastric cancer and pancreatic cancer. In gastric cancer, high expression of GABRP caused abnormal activation of the extracellular signal-regulated kinase (ERK) 1/2 signaling pathway while GABRP-mediated upregulation of the p38 pathway enhanced tumor metastasis [16]. In pancreatic cancer, GABRP activated the MAPK/ERK pathway by increasing Ca²⁺ levels to promote cell proliferation [17]. However, the functional role of GABRP played or whether it mediated drug resistance in CRC remains a mystery.

In summary, this study screened the GABRP molecule from three GEO datasets involved in CRC chemoresistance and GO annotation as well as KEGG pathway analysis. Next, we validated GABRP expression in our established chemoresistant cell lines (SW480 vs SW480-LOPH and HCT-8 vs HCT8-5Fu) both at the transcriptional level by the quantitative real-time polymerase chain reaction (qRT-PCR) and at

the protein level by the western blotting analysis. Interestingly, CD44 was also upregulated in chemo-resistant cells than their counterpart chemo-sensitive cells. It indicated different drug-resistant CRC cells induced CD44 expression which further strengthen the chemoresistance since CD44 is a well-known cancer stem cell marker. Eventually, the TCGA database also confirmed that GABRP was highly expressed in many cancer types, especially in CRC cancer patients. Moreover, GABRP levels were substantially upregulated in different types of colorectal cancer. CRC patients' tissues were stained with more GABRP proteins than normal colon tissues using the immunohistochemistry assay by the Human Protein Atlas (HPA) website. It suggested that GABRP can be considered as the diagnostic biomarker and may serve as a promising drug target to mediate chemoresistance in CRC. We illustrated the effect of the GABRP gene on CRC drug resistance and assessed its potential clinical value for treating advanced CRC patients.

Materials and methods

Identification of DEGs:

We downloaded three datasets including both drug-resistant and drug-sensitive samples from the Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/>) database with accession numbers of GSE28691, GSE77932, and GSE81006. GSE28691 characterized patients' derived oxaliplatin-sensitive tumors in contrast to parental human tumors in the NOD/SCID preclinical model of colorectal cancer. GSE77932 dataset compared DLD1 sensitive and its counterpart oxaliplatin-resistant cell lines, which contained six biological samples. GSE81006 compared the methylation status between HCT-8 wild-type cells (HCT8/WT) and its 5-Fu resistant cells (HCT8/5-Fu) for the time point at 72 h. Adjusted p-value < 0.01 or the absolute value of log₂Fold Change (FC) > 0.5 was set as the cut-off criteria to identify DEGs in colorectal sensitive and resistant cell lines. Then, DEGs had been analyzed and depicted utilizing the GEO2R online tool and the volcano plots, in which black dots represented none differentiated genes, red dots meant up-regulated genes while blue dots showed down-regulated genes. Overlapping DEGs were generated using the Venn Diagram (<http://bioinformatics.psb.ugent.be/webtools/Venn/>).

GO annotation and pathway signatures

Kyoto Encyclopedia of Genes and Genomes (KEGG, <http://www.genome.jp/>) is a knowledge database to identify DEGs and signaling pathways in relation to molecular interactive networks including cellular or metabolic processes, genetic or environmental effects, human disease, etc. The Database

for Annotation, Visualization, and Integrated Discovery (DAVID) online tool (<https://david.ncifcrf.gov/>) was applied to analyze the GO annotation and the KEGG pathway enrichments. In brief, we imported overlapping genes from three datasets (GSE28691, GSE81006, and GSE77932) into the DAVID online tool and identified the most frequently defined pathways. $P < 0.05$ is considered the statistical significance for the KEGG pathway.

Cell cultures

Human SW480 and HCT8 CRC cells were purchased from the Chinese Academy of Science (www.cellbank.org.cn) and authenticated by STR sequencing. The cells were cultured either in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma, Missouri, USA) or Roswell Park Memorial Institute (RPMI-1640) (Sigma, Missouri, USA) containing 10% FBS (fetal bovine serum) (Biological Industries, Kibbutz Beit-Haemek, Israel) supplemented with 1% penicillin/streptomycin antibiotics (Biosharp, Guangzhou, China) [18]. The CRC cells were incubated in the environment at 37 °C consisting of 5% CO₂, the cells were subcultured using 0.25% Trypsin–EDTA solution (Biosharp, Guangzhou, China).

Generation of the chemoresistant cell lines

SW480 and HCT8 cells in the logarithmic growth phase were inoculated and measured by cell viability assay to calculate the IC₅₀ value of oxaliplatin and 5-Fu [19]. SW480-Oxaliplatin (30 μM) and HCT-8-5Fu (20 μg/ml) resistant cells were established using an increasing concentration of these two drugs starting from slightly less than the IC₅₀ value of each drug for more than half a year. Cells were stimulated with incremental doses of different drugs over multiple cycles of weeks. The chemoresistance of each cell line has been validated by the cell viability assay [19].

qRT-PCR analysis

SW480, HCT8, and their counterpart chemoresistant cell lysates were harvested and homogenated. Then, the total RNA was harvested by TRIzol (Invitrogen, Carlsbad, CA) and extracted using chloroform, isopropanol precipitated RNA from the water phase followed by ethanol and centrifugation. The concentration of the total RNA was measured by Nano-one (Hangyan, Hangzhou, China) and displayed as the unit of ng/ul under the ratio of absorbance 260/280. 1.5–2 μg of total RNA reversed transcribed into 20 μl system using HiFiScript cDNA synthesis kit (CW BIO, Henan, China). qRT-PCR was conducted using SYBR® Green PCR Master Mix (Takara, Kusatsu, Japan). The PCR

reaction was monitored with Bio-Rad CFX 96 Touch Real-Time PCR Detection System (Bio-Rad, Shanghai, China). The thermocycling conditions for the PCR program were 50 °C for 2 min, 95 °C for 10 min, 40 cycles at 95 °C for 45 s, and the melting temperature was 55–65 °C for 30 s. All the experiments were independently performed at least in triplicates. The cyclic threshold (CT) of GABRP normalized to β-actin using $2^{-\Delta\Delta C_t}$ calculation methods [20]. All primers were listed below: β-actin forward primer: 5'-GACCAATCC TGTCACCTC-3', reversed primer: 5'-GATCTCCGTTCC CATTAAAGAG-3'; GABRP forward primer: 5'-GCCCTA ACAGAGCCTCAACA-3', reverse primer: 5'-TTGTCACTT CTGCCGACCTC-3'.

Western blotting analysis

The protein samples were boiled at 100 °C for 10 min. The concentration of quantified protein lysates was determined by the BCA protein assay (Vayzme biotech, Nanjing, China) and equal amounts of protein (6–12 μg) were loaded and separated by 8% polyacrylamide SDS-PAGE gels and then transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Missouri, USA). Next, the membranes were blocked with 5% non-fat milk for 1 h and incubated with primary antibodies according to the instructions and incubated with rotation at 4 °C fridges overnight, followed by 1 h secondary antibodies (anti-rabbit or anti-mouse immunoglobulin G, 1:2000, CWBIO, Henan, China). The protein bands were visualized by enhanced chemiluminescence ECL assay (Beoytime, Shanghai, China) and observed using Tannon 5200 Multi-functional machine. β-actin was used as the internal reference. The primary antibodies were listed below: GABRP (NOVUS, Colorado, USA), CD44 (cell signaling technology, Danvers, USA), and β-actin (Yeasen, Nanjing, China).

TCGA analysis

Boxplots were generated to compare the expression of GABRP among normal, CRC cancer patients and various cancer types of patients. $P < 0.05$ is considered significantly different for OncoPrint and TCGA analysis.

Immunohistochemistry analysis

The human protein atlas (HPA, <https://www.proteinatlas.org/>) is a protein database to achieve the protein distribution of human tissues and cells. HPA is composed of the immunohistochemical expression data among 20 common cancer types, classified with 12 subtypes of individual tumors.

GABRP protein expression in human normal and CRC tissues were downloaded and analyzed by HPA database.

Protein–protein interaction

The Search Tool for the Retrieval of Interaction Gene/Proteins (STRING) database (<https://string-db.org/>) was applied to compose the protein–protein interactive (PPI) network. “GABRP” molecule was imported as the protein name in the rectangular box, the organism was selected as Homo sapiens, and the possible network of protein interaction was generated. The middle circle displayed the hub gene GABRP connecting with other potential interactive proteins by the connecting lines. For example, every single gene was denoted by an individual circle, and the secondary structure of the protein was presented inside the circle. Furthermore, line numbers represented the strength of possible interactive molecules.

Statistical analysis

Student t-test and ANOVA analysis were utilized for all our data analysis. Hartley's test was used for the homogeneity of variances. All the experiments were repeated at least three times and performed independently, each experiment

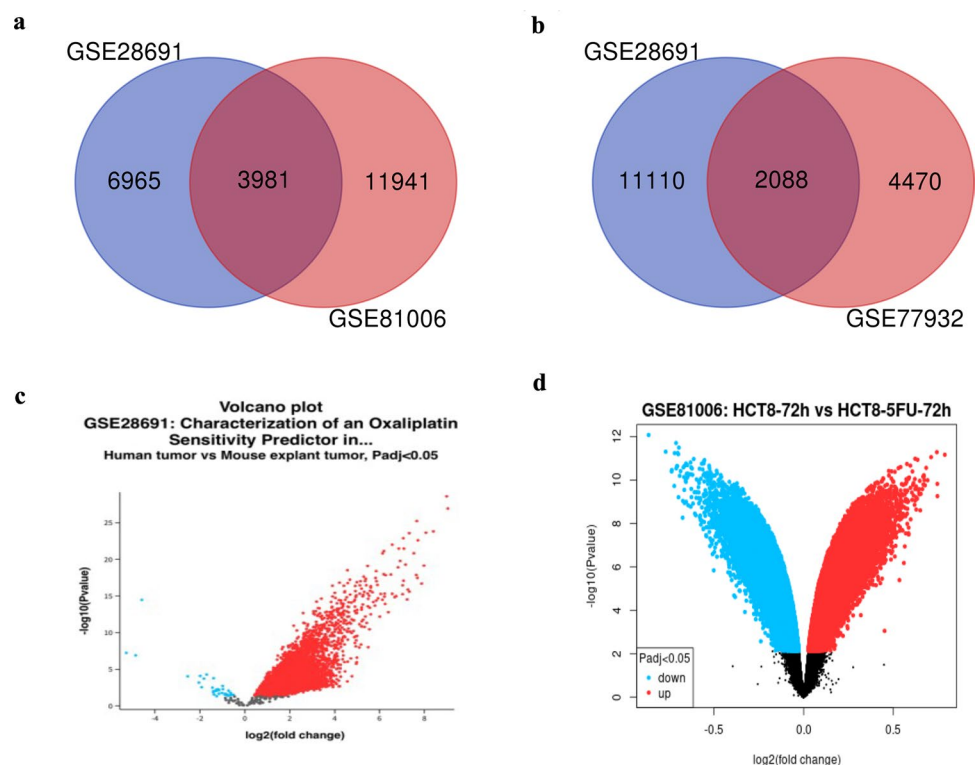
contained at least triplicates. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Results

Screening GABRP from human CRC chemo-resistant and sensitive patients' samples

In order to screen potential DEGs in chemo-resistant and chemo-sensitive colorectal patients, GSE28691, GSE77932, and GSE81006 datasets had been intersected. Firstly, GABRP stood out among 3981 overlapping genes from both GSE28691 and GSE81006 datasets with the selection condition of the adjusted p-value < 0.01 presented by the Venn diagram (Fig. 1a). Secondly, GABA family molecules had been enriched in the overlaps of GSE28691 and GSE77932 datasets, with the threshold setting as $|\log_2FC| > 0.5$ (Fig. 1b), we screened candidate genes such as GABARAPL1, GABRB1, GABRA3, GABRQ. Representative volcano plots of GSE28691 and GSE81006 datasets had been presented (Fig. 1c, 1d). Collectively, GABRP involved in the GABA family seems a promising differentiated gene between colorectal sensitive and resistant cells, therefore, we further decided to validate GABRP in two colorectal chemo-resistant and chemo-sensitive cancer cells.

Fig. 1 Screen of GABRP from human CRC chemo-resistant and sensitive patients' samples a. GABRP was selected from 3981 overlapping genes in the GSE28691 and GSE81006 data sets with an adjusted p-value < 0.01 b. The family of GABA molecules overlapped in the GSE28691 and GSE77932 datasets, the threshold is set to $|\log_2FC| > 0.5$ c. GABRP was up-regulated in the Volcano plot of DEGs between oxaliplatin-resistant and sensitive patients d. GABRP was up-regulated in the Volcano plot of DEGs between 5-Fu resistant and sensitive patients



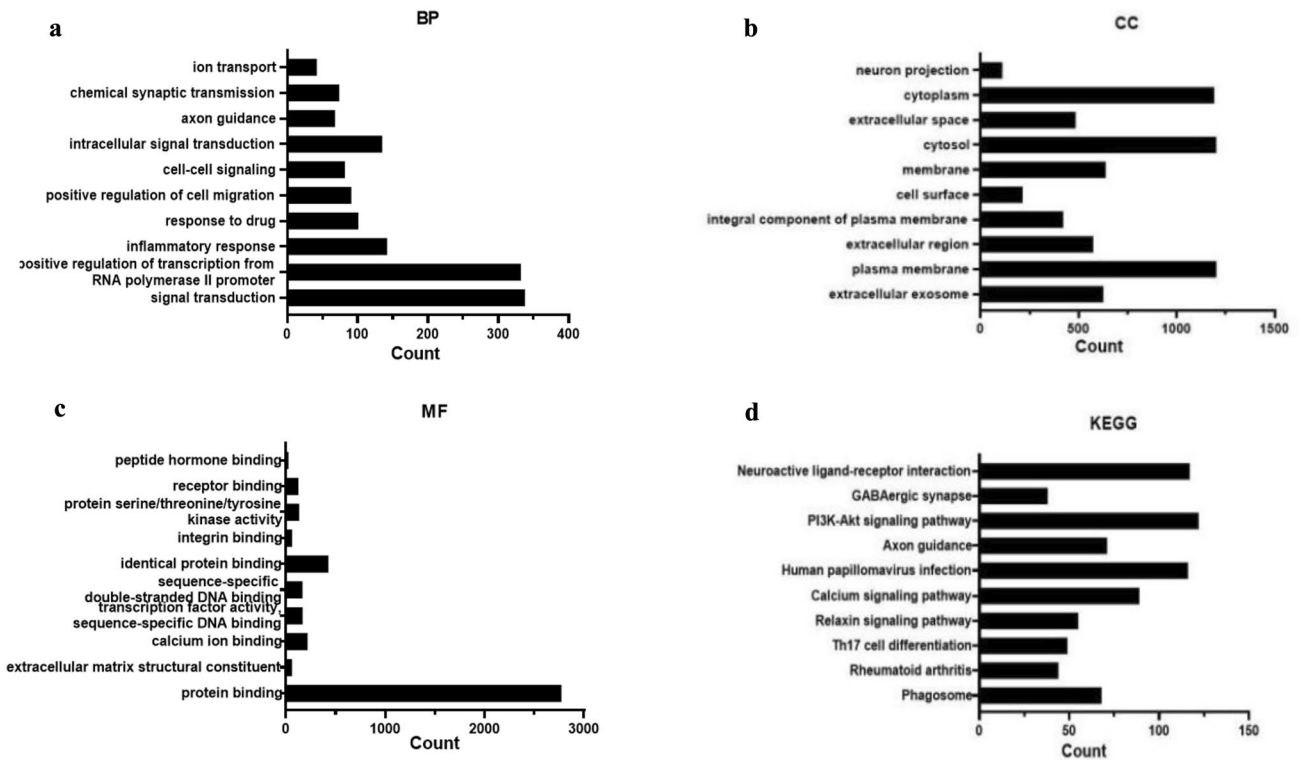


Fig. 2 KEGG pathway analysis and GO annotation a. GABRP was enriched in three biological processes: signal transduction, chemical synaptic transmission, and ion transport b. GABRP was enriched in four cellular components: plasma membrane, integral component of

plasma membrane, membrane, and neuron projection c. GABRP was enriched in two molecular functions: protein binding, and identical protein binding d. GABRP was present in the first two pathways of GABAergic synapse, and neuroactive ligand-receptor interaction

Table 1 Top ten genes by FDR in Biological Process

Term	Count	FDR
GO:0,007,165 ~ signal transduction	338	6.62E-11
GO:0,045,944 ~ positive regulation of transcription from RNA polymerase II promoter	332	6.62E-11
GO:0,006,954 ~ inflammatory response	142	6.62E-11
GO:0,042,493 ~ response to drug	101	1.37E-06
GO:0,030,335 ~ positive regulation of cell migration	91	1.42E-06
GO:0,007,267 ~ cell–cell signaling	82	5.01E-06
GO:0,035,556 ~ intracellular signal transduction	135	8.67E-06
GO:0,007,411 ~ axon guidance	68	1.22E-05
GO:0,007,268 ~ chemical synaptic transmission	74	9.42E-03
GO:0,006,811 ~ ion transport	42	4.83E-02

Table 2 Top ten genes by FDR in Cellular Components

Term	Count	FDR
GO:0,070,062 ~ extracellular exosome	626	4.74E-29
GO:0,005,886 ~ plasma membrane	1203	2.44E-27
GO:0,005,576 ~ extracellular region	575	2.88E-22
GO:0,005,887 ~ integral component of plasma membrane	422	8.65E-22
GO:0,009,986 ~ cell surface	215	2.90E-18
GO:0,016,020 ~ membrane	638	2.72E-17
GO:0,005,829 ~ cytosol	1202	1.46E-13
GO:0,005,615 ~ extracellular space	485	1.07E-12
GO:0,005,737 ~ cytoplasm	1192	1.32E-12
GO:0,043,005 ~ neuron projection	111	2.39E-06

KEGG pathway analysis and GO annotation

We imported 3981 genes into the DAVID database for GO and KEGG enrichment analysis. The results showed that the DEGs mainly participated in the biological process based on FDR ranking: such as signal transduction,

positive regulation of transcription from RNA polymerase II promoter, inflammatory response, response to drugs, positive regulation of cell migration, cell–cell signaling, intracellular signal transduction, axon guidance, chemical synaptic transmission, ion transport (Fig. 2a, Table 1). Our candidate gene GABRP was enriched in three biological processes: signal transduction, chemical synaptic transmission, and ion transport. Regarding cellular

components, the DEGs were mainly concentrated in the extracellular exosome, plasma membrane, extracellular region, integral component of the plasma membrane, cell surface, membrane, cytosol, extracellular space, cytoplasm, neuron projection, etc (Fig. 2b, Table 2). The gene of our interest GABRP was enriched in plasma membrane, integral component of plasma membrane, membrane, and neuron projection. In terms of molecular function, the DEGs were mainly participated in the protein binding, extracellular matrix structural, calcium ion binding, transcription factor activity, sequence-specific DNA binding, sequence-specific double-stranded DNA binding, identical protein binding, integrin binding, protein serine/threonine/tyrosine kinase activity, receptor binding, peptide hormone binding (Fig. 2c, Table 3). The GABRP was enriched in protein binding. KEGG pathway analysis enriched in phagosome, rheumatoid arthritis, Th17 cell differentiation, relaxin signaling pathway, calcium signaling pathway, human papillomavirus infection, axon guidance, PI3K-Akt signaling pathway, GABAergic synapse, and neuroactive ligand-receptor interaction (Fig. 2d, Table 4). Of note, the last two pathways listed in our representative bar graph contained our candidate gene GABRP.

Validation of GABRP expression between chemoresistant and sensitive CRC cells

GABRP mRNA expression increased in oxaliplatin-resistant SW480 cells more than two folds compared to the counterpart sensitive cells (Fig. 3a). 5-Fu resistant HCT-8 colorectal cells also detected an increment with one-and-a-half fold more GABRP expression from the transcriptional perspective (Fig. 3b). However, the protein level did not fully reflect the transcriptional level, GABRP protein was upregulated only in oxaliplatin-resistant SW480 CRC cells, while no significant increment of GABRP expression was robustly observed in 5-Fu-resistant HCT-8 CRC cells (Fig. 3c).

Table 3 Top ten genes by FDR in Molecular Function

Term	Count	FDR
GO:0,005,515 ~ protein binding	2774	1.85E-27
GO:0,005,201 ~ extracellular matrix structural constituent	61	2.43E-07
GO:0,005,509 ~ calcium ion binding	217	4.52E-07
GO:0,003,700 ~ transcription factor activity, sequence-specific DNA binding	169	8.18E-07
GO:1,990,837 ~ sequence-specific double-stranded DNA binding	166	5.75E-06
GO:0,042,802 ~ identical protein binding	426	6.64E-06
GO:0,005,178 ~ integrin binding	63	1.06E-05
GO:0,004,712 ~ protein serine/threonine/tyrosine kinase activity	133	1.1E-05
GO:0,005,102 ~ receptor binding	125	1.1E-05
GO:0,017,046 ~ peptide hormone binding	25	2.5E-05

Table 4 Top ten pathways by FDR in KEGG pathway analysis

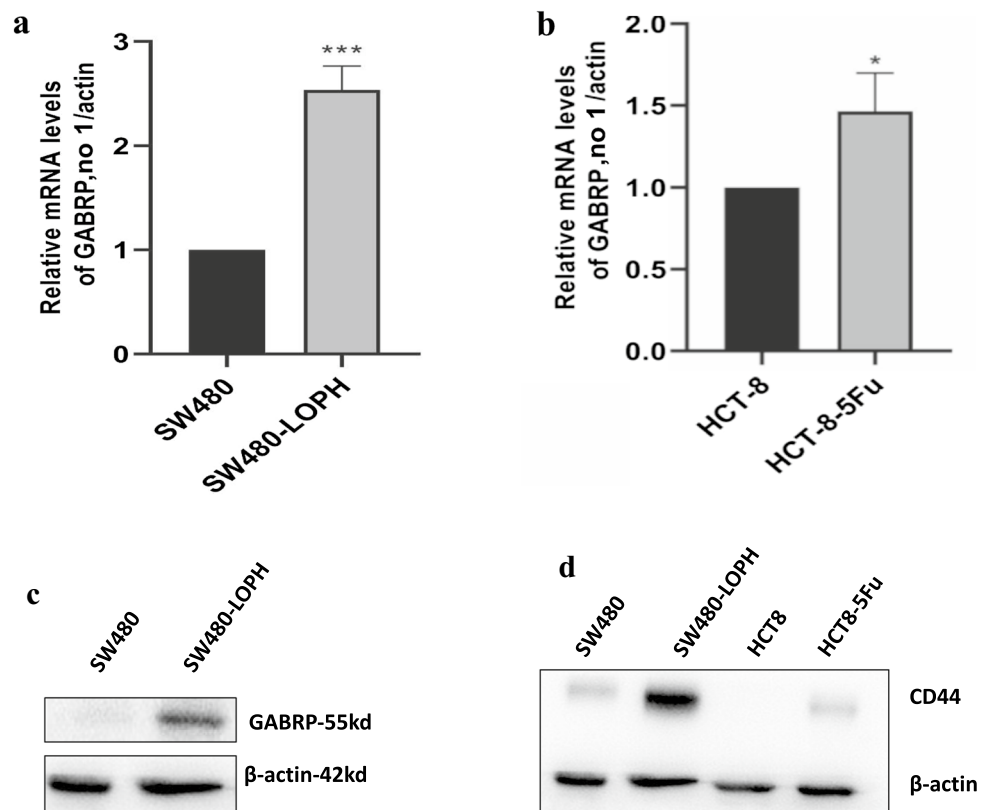
Term	Count	FDR
hsa04145:Phagosome	68	2.33E-05
hsa05323:Rheumatoid arthritis	44	3.69E-04
hsa04659:Th17 cell differentiation	49	3.69E-04
hsa04926:Relaxin signaling pathway	55	4.51E-04
hsa04020:Calcium signaling pathway	89	5.02E-04
hsa05165:Human papillomavirus infection	116	5.02E-04
hsa04360:Axon guidance	71	5.27E-04
hsa04151:PI3K-Akt signaling pathway	122	5.56E-04
hsa04727:GABAergic synapse	38	2.56E-03
hsa04080:Neuroactive ligand-receptor interaction	117	4.31E-03

Interestingly, both SW480-LOPH and HCT-8 5-Fu chemoresistant cells induced CD44 protein but not in their corresponding sensitive CRC cells (Fig. 3d), which suggested all the chemoresistant CRC was aggressive since CD44 was a well-known cancer stem cells marker, it can promote chemoresistance and metastasis.

Clinical relevance of GABRP in CRC patients

In order to further validate our findings in cancer patients in the clinical setting, we first compared GABRP expression among several cancer types such as breast cancer, colon cancer, gastric cancer, and pancreatic cancer. It was obvious that GABRP was overexpressed in colon cancer compared to the other three cancer types (Fig. 4a). Therefore, we switched gears to colorectal cancer patients. We found most colon and rectal cancer patients detected higher GABRP expression (Fig. 4b). Moreover, GABRP expression was upregulated for more than two folds in colorectal adenocarcinoma compared to the normal colorectal tissues (Fig. 4c). High GABRP protein level was detected in CRC patients' tissues but not obviously stained in normal tissues analyzed by the Human Protein Atlas (HPA) database (Fig. 4d). Collectively,

Fig. 3 GABRP expression between CRC cells and their chemoresistant cells a. High expression of GABRP mRNA in SW480-LOPH CRC cells b. High expression of GABRP mRNA in HCT-8-5Fu CRC cells c. High expression of GABRP protein in SW480-LOPH CRC cells d. High expression of CD44 protein in SW480-LOPH and HCT-8-5Fu CRC cells



consistent with our findings in the cell line models, clinical data supported the oncogenic role of GABRP expression in colorectal cancer patients.

Prediction of protein–protein interaction

In order to find interactive proteins with our candidate gene GABRP, we created its PPI network using the STRING database (Fig. 5a, b). GABRP was radiated as the hub gene in a plain PPI network with 10 circles and 17 lines connecting to it (Fig. 5a). There are ten proteins predicted to interact with GABRP: Ankyrin Repeat Domain 66 (ANKRD66), Clathrin Interactor 1 (CLINT1), Huntingtin Associated Protein 1 (HAP1), Phospholipase C Like 1 (Inactive) (PLCL1), GABA Type A Receptor-Associated Protein (GABARAP), GABA Type A Receptor Associated Protein Like 1 (GABARAPL1), N-Ethylmaleimide Sensitive Factor, Vesicle Fusing ATPase (NSF), GABA Type A Receptor Associated Protein Like 2 (GABARAPL2), Trafficking Kinesin Protein 2 (TRAK2), and Chloride Intracellular Channel 3 (CLIC3) (Fig. 5a). In this network, the average node degree was 3.09, the average local clustering coefficient was 0.896, and the PPI enrichment P-value was 0.0389. Moreover, we found that GABRP has a relatively stronger interaction with GABARAP, GABARAPL1, ANKRD66, CLINT1, and CLIC3 than the

above-mentioned molecules due to the indication of more line numbers (Fig. 5b). Therefore, we enriched highly interactive proteins in Fig. 5b. The average node degree was 2, the average local clustering coefficient was 0.85.

Discussion

Colorectal cancer (CRC) is one common and fatal cancer [21, 22]. Chemoresistance and metastatic factors contributed to the high mortality rate in colorectal cancer which its increment especially found in men or people under 50 years old [23]. The mechanism of chemoresistance is considered an intractable question. Our study successfully developed chemo-resistant cancer cells with elevated doses of oxaliplatin and 5-Fu, named SW480-LOPH, and HCT-8-5-Fu cell lines (Fig. 3). We found that the mRNA level of GABRP was significantly increased in oxaliplatin and 5-Fu chemoresistant cells than in those chemo-sensitive ones. Consistent with our results, JaeJin An et al. recently found that ligand GABA was critically decreased while its B subunit receptor GABABR expression was compensational upregulated in 5-FU-resistant-HT29 colorectal cancer cells [23]. The expression of GABABR1 was positively correlated with Galectin-3, which was related to the 5-Fu sensitivity in colon cancer cells [24].

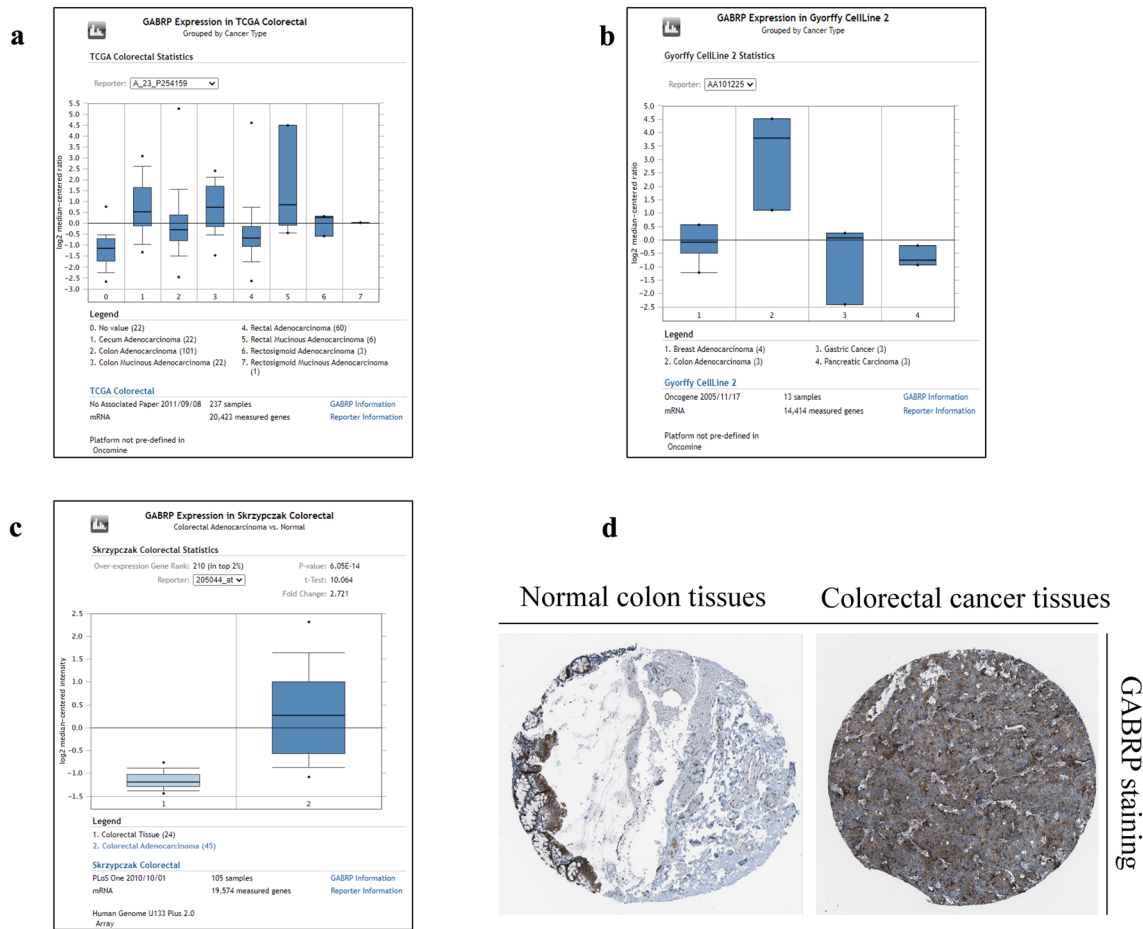
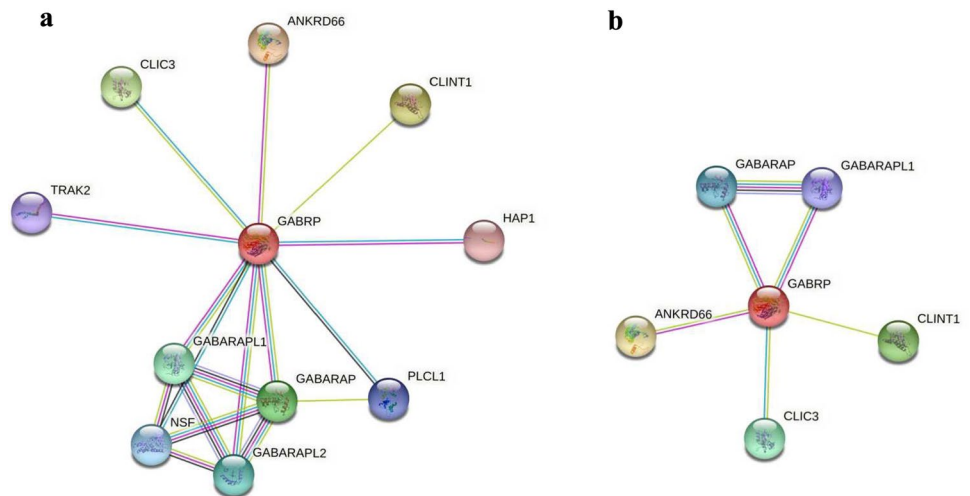


Fig. 4 Clinical relevance of GABRP in CRC patients a. GABRP overexpressed among patients with colorectal cancer tissues b. GABRP overexpressed in gastric cancer patients samples c. GABRP

upregulated in colorectal adenocarcinoma d. The expression of GABRP in colorectal cancer was significantly higher than that in normal tissues

Fig. 5 Protein–protein interaction networks a. 10 interactive proteins with the hub gene GABRP b. 5 enriched proteins with the hub gene GABRP



Interestingly, we found that GABARAP, GABARAPL1, and GABARAPL2 had relatively strong interactions with GABRP using the STRING database (Fig. 5). These

molecules may also be associated with colorectal cancer. Recent research proved that increased expression of GABARAP in colorectal cancer was significantly

associated with low differentiation and a relatively shorter overall survival rate [25]. Selenium affected autophagy by up-regulating GABARAPL1, which played a key role in the survival process of colorectal cancer cells. Depletion of GABARAPL1 promoted cell apoptosis in colorectal cancer [26]. Moreover, in colorectal cancer tissues, GABARAPL2 was the highest expressed gene among the subgroup of the autophagy-related genes which play a critical role in autophagosome elongation, and function in the regulation of receptor transport, cell proliferation, and autophagy [27]. Most predicted interactive molecules with GABRP by the STRING database exerted oncogenic characteristics in various cancer types. For example, GABARAP can participate in the autophagy of hepatocarcinoma (HCC) cells and endow cancer cells with radio-resistance [28]. High GABARAP expression was related to a poor prognosis of breast cancer [29]. While GABARAPL1 displayed the opposite tumor-suppressive role in breast cancer [30].

Given that GABRP and its family proteins were all abnormally expressed in multiple cancer types such as gastric cancer, breast cancer, pancreatic cancer, ovarian cancer etc. [16], the mechanism of GABRP-mediated chemo-resistance in colorectal cancer is still unknown. The loss-of-function study displayed that silence or pharmacological inhibition of GABRP downregulated Erk1/2 expression which resulted in reducing cell proliferation, migration, invasion, and metastatic potential [16]. These findings suggested that GABRP may play an indispensable role in chemo-resistance in many cancers. Our study revealed that GABRP was highly expressed in oxaliplatin/5-Fu-resistant colorectal cancer cells, which developing novel targets are in urgent need to improve the therapeutic outcomes of colorectal cancer patients. The expression of GABRP in triple-negative breast cancer (TNBC) cells was upregulated and it promoted tumor growth both in vitro and in vivo. It can also be used as a new target for breast cancer therapy in the future [31]. Anti-GABRP-ADC may have therapeutic effects on colorectal cancer, triple-negative breast cancer, and other cancers [31]. We found both oxaliplatin and 5-Fu-resistant CRC cells enhanced CD44 protein expression, and GABRP proteins were robustly increased in SW480-LOPH cells but not in HCT-8-5Fu CRC cells which suggested SW480-LOPH cells maybe more malignant. Coincidentally, we also found CD44 protein level was significantly higher in SW480-LOPH cells than it in HCT-8-5Fu cells. Our group identified GABRP molecule played an important role in mediating gemcitabine-treated pancreatic cancer cells, shRNA stable knock-down CD44 decreased cell viability and decreased GABRP expression at the transcriptional level in pancreatic cancer as well. Our CFPAC-1-gemcitabine-resistant pancreatic cancer cells had more CD44 proteins than CFPAC-1 sensitive cells [32]. We speculated various drugs may target different

downstream proteins, Oxaliplatin and Gemcitabine but not 5-Fu may target the GABRP molecule, current literature still supported our results that GABRP was positively associated with chemoresistant CRC cells. In summary, GABRP may be identified as a promising target for reversing chemoresistance in a variety of cancer treatments, especially in colorectal cancer.

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Author contributions ZH, CC, and JC contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by TW, QZ, TW, LJ, SY, and YF. The first draft of the manuscript was written by TW, QZ and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. TW and QZ contributed equally to this paper.

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Declarations

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