Downregulation of B3GNT6 is a predictor of poor outcomes in patients with colorectal cancer

Shihan Xiao1,2,3†, Chen Yang1,4†, Yang Zhang1,2,3 and Chen Lai1,2,3*

Abstract
Background: The B3GNT6 protein is a member of the O-GlcNAc transferase (OGT) family and is responsible for the production of the core 3 structure of O-glycans. It is generally expressed in the gastrointestinal (GI) tract; however, its clinical significance in colorectal cancer remains largely unexplored.

Methods: We obtained mRNA transcriptomic sequencing data from 3 gene expression omnibus (GEO) datasets (GSE37182, GSE39582, GSE103512) and The Cancer Genome Atlas (TCGA) to compare the B3GNT6 mRNA levels between colorectal cancer and normal tissues and further evaluate its value as a prognostic marker in colorectal cancer. We further validated this at the protein level in our cohort using immunohistochemical staining of B3GNT6 as well as the Human Protein Atlas online database.

Results: B3GNT6 expression was downregulated in colorectal cancer tissues as compared to that in the normal tissues at both mRNA and protein levels. Downregulation of B3GNT6 expression was found to be associated with poor overall survival in patients with colorectal cancer as per the data in GSE39582 and TCGA databases. Low B3GNT6 mRNA levels were significantly associated with chromosome instability (CIN) and KRAS mutations in patients with colorectal cancer. Gene set enrichment analysis (GSEA) revealed that low B3GNT6 expression levels in colorectal cancer were associated with increased proteasome activity.

Conclusions: The results of this study demonstrate that low expression of B3GNT6 is a potential biomarker for poor outcomes in patients with CRC. Moreover, the low expression of B3GNT6 may indicate more frequent activation of the KRAS/ERK signaling pathway, high CIN, and increased proteasomal activity. These novel findings may prove helpful for molecular diagnosis and provide a new therapeutic target for colorectal cancer.

Keywords: B3GNT6, Bioinformatics analysis, TCGA, GSEA, Proteasome

Introduction
Colorectal cancer accounts for nearly 900,000 deaths annually and is ranked as the world's 4th most deadly malignancy [1]. Though recent advances in treatment options have almost doubled the overall survival in patients with advanced disease to 3 years, the prognosis is still the best for those with non-metastasized disease [2]. Thus, it is imperative to identify novel prognostic biomarkers for patients with colorectal cancer.

Colorectal cancer is known to comprise various types of genomic mutations that ultimately lead to different prognoses. Chromosome instability (CIN), microsatellite instability (MIN), and CpG island methylator phenotype (CIMP) pathways are the three major well-known molecular pathways in colorectal cancer; each pathway entails
varying histology, risk factors, prognosis, and response to therapy [3]. The gene mutations associated with these molecular pathways largely affect patient response to therapy. Herein, prognostic and predictive biomarkers may enable proficient management of colorectal cancer.

The B3GNT6 protein, also known as core 3 synthase, is a member of the O-GlcNAc transferase (OGT) family and adds an N-acetylglucosamine to N-acetylgalactosamine-modified serine or threonine. The B3GNT6 protein is responsible for the formation of the core 3 structure of O-glycans, which are important components of mucin-type glycoproteins [4]; this process is known as the protein O-GlcNAcylation modification. O-glycans are often mutated or dysregulated in structure in various types of cancers and greatly contribute to the abnormal biological activities seen in cancer cells [5]. Numerous studies have reported that aberrant protein O-GlcNAcylation plays a key role in the growth, metastasis, and progression of many malignant tumors [6–9]. The B3GNT protein family is commonly dysregulated in various types of cancers, including gastrointestinal tumors, cervical cancer, and prostate cancer [9, 10]. B3GNT6 expression has been reported to be largely downregulated in gastric and colorectal cancers [10]. However, the underlying mechanism and relationship between B3GNT6 expression and cancer progression and metastasis remain largely unexplored.

The ubiquitin-proteasome system (UPS) plays a pivotal role in the growth and survival of cancer cells. It is a major component of the cellular protein degradation machinery, which allows the degradation of misfolded and dysregulated proteins in the cells. As an important regulator of a variety of protein substrates, the proteasome contributes virtually to every cellular function, including proliferation, apoptosis, angiogenesis, and metastasis [11]. The proper function of the cellular proteasome is crucial for the survival of both normal and neoplastic cells, especially when the apoptosis proteasome is inhibited. Understanding the function and control of the ubiquitin-proteasome system will further illustrate the regulation of cellular activity and tumor progression.

Herein, we conducted an in-depth bioinformatics analysis using online databases to investigate the mRNA and protein expression patterns of B3GNT6 and its clinical significance in colorectal cancer. Immunohistochemical analysis of B3GNT6 protein expression was also conducted in 43 paired tissues from our own cohort of colorectal cancer patients.

Our results demonstrated that B3GNT6 was downregulated in colorectal cancer tissues as compared with normal tissues at both mRNA and protein levels. B3GNT6 mRNA expression levels were negatively correlated with overall survival in patients with colorectal cancer. B3GNT6 levels in colorectal cancer patients were associated with CIN and KRAS mutation status. Bioinformatic analyses revealed that downregulation of B3GNT6 expression is enriched in the proteasome pathway.

Materials and methods
Data collection and processing
Three datasets including gene expression data of colorectal cancer patients were downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). Datasets GSE39582 [12], GSE103582 [13], and GSE37182 [14] contained transcriptomic mRNA expression profiles of 566, 50, and 88 colorectal cancer cases with 19, 84, and 10 normal cases, respectively. Level 3 HTSeq-FPKM files, comprising 612 transcriptome profiling RNA-Seqs of 544 cases, were collected from a dataset in TCGA (https://portal.gdc.cancer.gov/) with information on 452 and 96 patients with colon and rectal cancers, respectively. The clinicopathological characteristics, such as age, sex, clinical TNM stage, CIN, MMR, KRAS, and BRAF mutation status were included. The expression levels of the B3GNT6 gene in other cell lines, organs, and cancers were obtained from the MediSapiens IST online database (http://ist.medisapiens.com/). The protein expression levels of B3GNT6 were reviewed using immunohistochemical-staining data provided in the Human Protein Atlas (http://www.proteinatlas.org/).

Gene set enrichment analysis (GSEA)
To determine the function of B3GNT6, we conducted GSEA using samples with top 25% and bottom 25% of B3GNT6 expression in the GSE39582 dataset with the GSEA 4.1.0 software (https://www.gsea-msigdb.org/gsea). The annotated gene sets c2.cp.kegg.v6.0.symbols.gmt and c2.cp.biocarta.v6.0.symbols.gmt from the pathway database were used as the reference gene sets. The cutoff criteria used were as follows: p < 0.05, |enrichment score (ES)| > 0.3, and gene size ≥ 30.

Immunohistochemical staining to detect B3GNT6 protein expression in colorectal cancer patients
We obtained 43 cancer tissues, paired adjacent non-tumor tissues, and related clinical information from patients who underwent radical colorectal resection in the Department of General Surgery, Xiangya Hospital of Central South University, between February 2016 and July 2018. All tissues collected were clinically and pathologically diagnosed as colorectal cancer. Patients with recurrence or those who had received adjuvant chemotherapy or radiotherapy were excluded from our study. The differential expression levels of B3GNT6 protein in 43
colorectal cancer and paired normal tissues were measured using IHC staining using previously established protocols [15]. Rabbit polyclonal anti-B3GNT6 (21291-1-AP, Thermofisher, US) was used at a working dilution of 1:100 co. The scores were evaluated on the basis of staining intensity and percentage of positive cells for each section. The staining intensity was scored as follows: 0, no staining; 1, light yellow staining; 2, yellow-brown staining; and 3, deep brown staining. The percentage of positive cells was scored as follows: 0, 0~5%; 1, 6~25%; 2, 26~50%; 3, 51~75%; and 4, > 75%. The final score was calculated using the following formula: positive cell score $\times$ staining intensity score. The total scores were condensed into four categories: 0 for negative (−), 1–3 for weakly positive (+), 4–7 for positive (++), and 8–12 for strongly positive (+++). All patients were sorted into two groups according to the total scores. High expression of B3GNT6 protein was defined as a detectable immunoreaction with a total score of ≥1+.

**Statistical analysis**

Statistical analyses were performed using R studio (version 1.3.1056) and Graphpad Prism (Version 8.0.2). The comparison between B3GNT6 mRNA expression levels in colorectal cancer and normal tissues from TCGA and GEO databases was performed using unpaired Student’s t tests. The diagnostic value of B3GNT6 mRNA expression was evaluated using a receiver operating characteristic (ROC) curve. Survival analysis was conducted using log-rank (Mantel-Cox) test. The association between clinicopathological characteristics and B3GNT6 mRNA expression levels was determined using the $\chi^2$ test. $p < 0.05$ was considered statistically significant.

**Results**

**B3GNT6 mRNA expression is downregulated in colorectal cancer tissues**

To measure B3GNT6 mRNA levels in colorectal cancer tissues, we first analyzed B3GNT6 mRNA levels by comparing mRNA levels in the tumor tissues compared with that in normal or para-tumor tissues in GSE37182, GSE39582, and GSE103512. B3GNT6 mRNA levels were significantly lower in tumor tissues than those in normal tissues in all three microarrays. This was further validated using the TCGA online database (Fig. 1).
area under the curve (AUC) of the four ROC curves was all greater than 0.6, demonstrating a good diagnostic value. The GSE39582 dataset showed the highest AUC (0.9508); when the cut-off value was <6.533, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 92.76%, 94.74%, 94.6%, and 93%, respectively. The accuracy was the highest, at 93.75%. To analyze B3GNT6 mRNA levels in other organs, we used the IST Online database (http://ist.medisapiens.com/). Results indicated that in the GI tract, the stomach, esophagus, and normal colorectal tissues, B3GNT6 mRNA levels were higher than those in colorectal cancer tissues; however, the small intestine showed low B3GNT6 expression. B3GNT6 mRNA levels were also higher in the bronchus (Supplementary Fig. 1).

**Correlation between B3GNT6 mRNA levels and clinicopathological characteristics in the GSE39582 dataset**

We then focused on the GSE39582 dataset, which had the largest sample size and the most detailed information (Table 1). The dataset comprised two independent cohorts, the testing cohort \( n = 443 \) and validating cohort \( n = 123 \). Subgroup analysis showed that the chromosome instability (CIN) and KRAS mutated groups had patients with lower B3GNT6 mRNA levels.

**B3GNT6 protein expression is downregulated in colorectal cancer tissues**

To further address the change in B3GNT6 expression and its clinical significance in patients with colorectal cancer, we analyzed the online databases of protein expression together with our own clinical cohorts. We downloaded and analyzed B3GNT6 immunohistochemical micrographs in colorectal cancer and normal colon tissues from the Human Protein Atlas (https://www.proteinatlas.org/). Negative (11/12) or low (1/12) staining of B3GNT6 protein was observed in colorectal cancer tissues when compared to the medium staining in normal colon (3/3) and rectal tissues (3/3) (Fig. 2A, B, E, and F). The staining of B3GNT6 protein was largely localized to the cytoplasmic area, supposedly in the Golgi apparatus area [10]. We also conducted immunohistochemical analyses in 43 colorectal cancer tissues with paired adjacent non-tumor tissues, which also showed downregulation of B3GNT6 protein expression in colorectal cancer. Negative (5/43) or weakly positive (30/43) or positive (8/43) staining of B3GNT6 protein was observed in colorectal cancer tissues when compared to the weakly positive (5/43) or positive (27/43) staining or strongly positive (11/43) staining in paired adjacent non-tumor tissues (Fig. 2C, D, G, and H and Fig. 3).

**Upregulation of B3GNT6 expression is associated with better overall survival in patients with colorectal cancer**

Next, we examined B3GNT6 expression with regard to its prognostic significance in colorectal cancer. In the GSE39582 dataset, patients with high B3GNT6 levels showed better overall survival than those with low B3GNT6 levels. This was further validated using the TCGA dataset (Fig. 4A–C). Together, these findings indicate that high B3GNT6 expression has clinical significance and could potentially serve as an important biomarker for predicting clinical outcomes in patients with colorectal cancer.

**Downregulation of B3GNT6 expression is correlated with upregulated proteasome activity**

Based on the abovementioned analysis, it is likely that B3GNT6 might act as a tumor suppressor in the colorectal cancer microenvironment. To better understand the mechanism underlying the role of B3GNT6 as a tumor suppressor, gene set enrichment analysis (GSEA) was conducted in the GSE39582 dataset. The top 25% and bottom 25% B3GNT6 mRNA levels in patients enrolled in this study were used for GSEA. We adopted both KEGG and Biocarta pathway analyses in GSEA, and both pathway analyses indicated that B3GNT6 mRNA levels were negatively correlated with the ubiquitin-proteasome system (Table 2, Fig. 5, and Supplementary Fig. 2).

**Discussion**

We conducted a bioinformatic analysis of GEO and TCGA datasets as well as our own cohort and found that the mRNA and protein levels of B3GNT6 in colorectal cancer tissues were significantly lower than those in the normal tissues, which was consistent with the results of previous studies [10]. Survival analysis showed that the downregulation of B3GNT6 mRNA expression was associated with a low overall survival rate of patients with colorectal cancer. The downregulation of B3GNT6 mRNA expression in colorectal cancer tissues is related to CIN status, KRAS mutation, and proteasome pathway. Our study found that colorectal cancer patients with low B3GNT6 levels are more likely to develop KRAS mutations and chromosomal instability (CIN). This is interesting because it may clarify the role of B3GNT6 expression in the development of colorectal cancer. The chromosomal instability (CIN) pathway is usually driven by sequence mutation events, the most common being...
Table 1  Association between B3GNT6 level and clinicopathological factors from dataset GSE39582

| Variables                      | Testing cohort (n=443) | Validating cohort (n=123) |
|--------------------------------|------------------------|---------------------------|
|                                | High (n=170)          | Low (n=273)               | p value       | High (n=49) | Low (n=74) | p value       |
| Age                            |                        |                           |               |             |             |               |
| ≥65                            | 101                    | 166                       | 0.7351        | 35          | 52          | 0.8901        |
| <65                            | 69                     | 106                       |               | 14          | 22          |               |
| Unavailable                     | 0                      | 1                         |               | 0           | 0           |               |
| Gender                         |                        |                           |               |             |             |               |
| Male                           | 90                     | 147                       | 0.8527        | 32          | 41          | 0.2738        |
| Female                         | 80                     | 126                       |               | 17          | 33          |               |
| Stage                          |                        |                           |               |             |             |               |
| I-II                           | 84                     | 137                       | 0.9321        | 28          | 48          | 0.3882        |
| III-IV                         | 82                     | 136                       |               | 21          | 26          |               |
| T stage                        |                        |                           |               |             |             |               |
| T1-T2                          | 21                     | 26                        | 0.3574        | 2           | 11          | 0.0625        |
| T3-T4                          | 145                    | 239                       |               | 43          | 59          |               |
| Unavailable                     | 4                      | 8                         |               | 4           | 4           |               |
| Lymph node metastasis          |                        |                           |               |             |             |               |
| Absent                         | 88                     | 142                       | 0.8803        | 26          | 46          | 0.3906        |
| Present                        | 76                     | 119                       |               | 19          | 24          |               |
| Unavailable                     | 6                      | 12                        |               | 4           | 4           |               |
| Distant metastasis             |                        |                           |               |             |             |               |
| Absent                         | 150                    | 226                       | 0.1474        | 40          | 66          | 0.3078        |
| Present                        | 16                     | 38                        |               | 4           | 3           |               |
| Unavailable                     | 4                      | 9                         |               | 5           | 5           |               |
| Tumor Location                 |                        |                           |               |             |             |               |
| Distal                         | 96                     | 171                       | 0.1971        | 27          | 48          | 0.2772        |
| Proximal                       | 74                     | 102                       |               | 22          | 26          |               |
| MMR               |                        |                           |               |             |             |               |
| dMMR                           | 18                     | 43                        | 0.1222        | 5           | 9           | 0.6202        |
| pMMR                           | 139                    | 209                       |               | 41          | 55          |               |
| Unavailable                     | 13                     | 21                        |               | 3           | 10          |               |
| CIMP status                     |                        |                           |               |             |             |               |
| −                              | 111                    | 195                       | 0.1855        | 38          | 61          | 0.4994        |
| +                              | 33                     | 41                        |               | 8           | 9           |               |
| Unavailable                     | 26                     | 37                        |               | 3           | 4           |               |
| CIN status                      |                        |                           |               |             |             |               |
| negative                       | 41                     | 41                        | 0.0205        | 17          | 11          | 0.0059        |
| positive                       | 98                     | 176                       |               | 25          | 55          |               |
| Unavailable                     | 31                     | 56                        |               | 7           | 8           |               |
| tp53 mutation                  |                        |                           |               |             |             |               |
| Mutated                        | 51                     | 84                        | 0.9483        | 20          | 35          | 0.2642        |
| Wild type                      | 42                     | 68                        |               | 24          | 27          |               |
| Unavailable                     | 77                     | 121                       |               | 5           | 12          |               |
| Kras mutation                  |                        |                           |               |             |             |               |
| Mutated                        | 84                     | 88                        | 0.0002        | 20          | 25          | 0.496         |
| Wild type                      | 78                     | 174                       |               | 29          | 47          |               |
| Unavailable                     | 8                      | 11                        |               | 0           | 2           |               |
| Braf mutation                  |                        |                           |               |             |             |               |
| Mutated                        | 19                     | 25                        | 0.4532        | 4           | 3           | 0.3401        |
| Wild type                      | 130                    | 218                       |               | 44          | 69          |               |
| Unavailable                     | 21                     | 30                        |               | 1           | 2           |               |

*MMR mismatch repair, dMMR mismatch repair-deficient, pMMR mismatch repair-proficient, CIMP CpG island methylator phenotype, CIN chromosome instability*
KRAS, which leads to typical aneuploid microsatellite stable (MSS) colorectal cancer, and the sequence mutation event that drives CIN is more likely to be KRAS than BRAF [16]. The results of bioinformatics analyses showed that the levels of B3GNT6 mRNA expression in the KRAS mutation group were lower. We speculated that B3GNT6 might negatively regulate the occurrence and metastasis of colorectal cancer through the KRAS/ERK signal pathway. Previous research supports this hypothesis that the KRAS/ERK signaling pathway is closely related to OGT levels in pancreatic, gastric, and cervical cancers [17–19].

The B3GNT6 protein is a key enzyme in the synthesis of the core 3 structure of O-glycans, and its low expression or non-expression causes aberrant protein O-GlcNAcylation. A study by Radhakrishnan et al. revealed that the expression of the core 3 structure of O-glycans affects the production and stability regulation of α1 and β2 integrin in pancreatic cancer, thus inhibiting metastasis [6]. Boottanun and colleagues reported that the low expression of B3GNT6 is associated with higher differentiation and better prognosis in patients with cholangiocarcinoma [7]. In prostate cancer, B3GNT6 inhibits tumor formation and metastasis through the downregulation of the α2-β1 integrin complex [9]. Li et al. reported that α1 integrin enhanced tumorigenicity and invasiveness of colorectal cancer. It is worth noting that this mechanism acts through the activation of the KRAS/ERK signaling pathway [8]. This may confirm our hypothesis that the B3GNT6 protein regulates the KRAS/ERK signaling pathway. Further molecular biological studies are warranted to validate the regulatory effect of B3GNT6 expression on the KRAS signaling pathway.

Our results also showed that the downregulation of B3GNT6 mRNA expression levels is related to increased proteasome activity. This is interesting because proteasomes often form a proteasome system.
(UPS) with ubiquitin. Under normal physiological conditions, the UPS is responsible for eliminating dysfunctional/misfolded proteins through the proteasome. These specific functions enable the UPS to regulate protein quality in cells, thus maintaining cellular homeostasis and cell survival [20]. Dysregulation of the UPS is commonly observed in various cancers, and its dysfunction may promote tumor development by regulating the degradation of specific proteins [21, 22]. Over the past decade, the United States Food and Drug Administration has approved a few UPS inhibitors for hematological malignant tumors and these drugs have already achieved curative effects in a few cases. However, due to the characteristics of the proteasome itself, the development of drugs based on protein degradation is rather slow. Presently, the practice of UPS inhibitors in the field of colorectal cancer and other solid tumors is still in the exploratory stage, and the limited clinical remission rate makes it not put into clinical application on a large scale for the time being [23–25].

Our understanding of the specific mechanism of UPS remains limited, but given the results of previous studies and ours [22, 25], we consider that the increased activity of UPS promotes the occurrence and metastasis of colorectal cancer and eventually leads to a poor prognosis for patients. In tumor environments, downregulation of B3GNT6 expression may lead to upregulation of proteasome activity, which in turn suppresses the accumulation of misfolded or toxic proteins in tumor cells. This disrupts the apoptosis of these tumor cells that eventually results in tumor formation. Further well-designed studies focusing on the role of B3GNT6 in proteasome regulation are necessary because their association has not been reported as yet.

Fig. 4 Increased B3GNT6 expression predicts better overall survival in patients with colorectal cancer. A, B Both the discovery and validation cohorts in GSE39582 comprised patients with high B3GNT6 mRNA levels showed better overall survival. C This was further validated using clinical data from TCGA online database.
Conclusions
The findings of this study demonstrate that low B3GNT6 expression is a potential biomarker of poor outcomes in patients with CRC and may lead to more frequent activation of the KRAS/ERK signaling pathway, CIN, and increased proteasomal activity. These new results may prove helpful in the molecular diagnosis of CRC as well as the creation of a new therapeutic target for CRC. Further confirmation using a large and multi-center clinical cohort is required for the validation of its predictive ability. Further laboratory diagnosis should be undertaken to verify the molecular mechanism underlying the relationship between B3GNT6 levels and KRAS mutation, CIN, and increased proteasomal activity.

Table 2: GSEA analysis of B3GNT6 mRNA expression in GSE39582

| Geneset name | NES  | NOM p-val | FDR q-val |
|--------------|------|-----------|-----------|
| BIOCARTA pathway | Upregulated | | |
| BIOCARTA_RAB_PATHWAY | 1.6703635 | 0.026 | 0.33836955 |
| BIOCARTA_CERAMIDE_PATHWAY | 1.6385397 | 0.03807615 | 0.14160849 |
| Downregulated | | | |
| BIOCARTA_PROTEASOME_PATHWAY | -1.865893 | 0.01123595 | 0.15191999 |
| KEGG pathway | Upregulated | | |
| KEGG_OLFACTORY_TRANSDUCTION | 2.0274692 | 0 | 0.027611418 |
| KEGG_TASTE_TRANSDUCTION | 1.7740936 | 0 | 0.19784024 |
| KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_LACTO_AND_NEOLACTO_SERIES | 1.770417 | 0.001926782 | 0.13764085 |
| KEGG_O_GLYCAN_BIOSYNTHESIS | 1.7251008 | 0.007736944 | 0.16378903 |
| KEGG_GNRH_SIGNALING_PATHWAY | 1.7178557 | 0 | 0.14160849 |
| KEGG_ALZHEIMERS_DISEASE | 1.6913323 | 0.026923077 | 0.15125382 |
| KEGG_NITROGEN_METABOLISM | 1.6708751 | 0.001992032 | 0.13392551 |
| KEGG_LONG_TERM_POTENTIATION | 1.64782 | 0.024528302 | 0.14832915 |
| KEGG_HUNTINGTONS_DISEASE | 1.638019 | 0.04660194 | 0.14545798 |
| KEGG_VASOPRESSIN_REGULATED_WATER_REABSORPTION | 1.63744 | 0.018072288 | 0.1325422 |
| KEGG_BUTANOATE_METABOLISM | 1.6251645 | 0.01778656 | 0.13560429 |
| KEGG_DRUG_METABOLISM_OTHER_ENZYMES | 1.6193944 | 0.01178782 | 0.13172121 |
| KEGG_PORPHYRIN_AND_CHLOROPHYLL_METABOLISM | 1.5756354 | 0.025742574 | 0.17885368 |
| KEGG_MATURITY_ONSET_DIABETES_OF_THE_YOUNG | 1.5706768 | 0.027613413 | 0.17375107 |
| KEGG_TERPENOID_BACKBONE_BIOSYNTHESIS | 1.5477382 | 0.046092186 | 0.19446045 |
| KEGG_AMYOTROPHIC_LATERAL_SCLEROSIS_ALS | 1.5473746 | 0.02591813 | 0.13857272 |
| KEGG_GLYCEROPHOSPHOLIPID_METABOLISM | 1.5310374 | 0.015873017 | 0.1866673 |
| KEGG_RETINOL_METABOLISM | 1.526893 | 0.02970297 | 0.18431947 |
| KEGG_GLYCOLYSIS_GLUCONEOGENESIS | 1.5237751 | 0.038 | 0.1788556 |
| KEGG_VIBRIO_CHOLERAE_INFECTION | 1.5188705 | 0.020876827 | 0.17640822 |
| KEGG_PROXIMAL_TUBULE_BICARBONATE_RECLAMATION | 1.4940959 | 0.048543688 | 0.20143263 |
| KEGG_STARCH_AND_SUCROSE_METABOLISM | 1.4879901 | 0.037848607 | 0.20171466 |
| KEGG_ASCORBATE_AND_ALDARATE_METABOLISM | 1.4647686 | 0.03976143 | 0.21592942 |
| KEGG_BASAL_TRANSCRIPTION_FACTORS | -1.7751815 | 0.009920635 | 0.3991878 |
| KEGG_UBIQUITIN_MEDIATED_PROTEOLYSIS | -1.773844 | 0.013861386 | 0.20233637 |
| KEGG_RNA_DEGRADATION | -1.6950728 | 0.026868524 | 0.2168877 |
| KEGG_GLYCOSAMINOLIGCAN_BIOSYNTHESIS_HEPARAN_SULFATE | -1.5696611 | 0.020715632 | 0.3765442 |
| KEGG_GLYCOSAMINOLIGCAN_BIOSYNTHESIS_CHONDROITIN_SULFATE | -1.5518181 | 0.04183267 | 0.3753312 |

NES normalized enrichment score, that is, the enrichment score for the gene set after it has been normalized across analyzed gene sets. FDR q-val false discovery rate, that is, the estimated probability that the normalized enrichment score represents a false positive finding. NOM p-val normalized p value, that is, the statistical significance of the enrichment score. The nominal p value is not adjusted for gene set size or multiple hypothesis testing; therefore, it is of limited use in comparing gene sets.
Fig. 5 GSEA indicates low B3GNT6 levels are associated with increased proteasome activity. **A** The butterfly plot shows the positive and negative correlation between gene rank and the ranking metric score. **B** The enrichment plot indicates that B3GNT6 levels are negatively correlated with proteasome activity in the KEGG pathway.
Abbreviations
CIN: Chromosome instability; MIN: Microsatellite instability; CIMP: CpG island methylator phenotype; MSS: Microsatellite stable; B3GNT6: UDP-GlcNAc:BetaGal Beta-1,3-N-acetylgalcosaminyltransferase 6; GEO: Gene Expression Omnibus; TCGA: The Cancer Genome Atlas; UPS: Ubiquitin-proteasome system; ROC: Receiver operating characteristic.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12957-022-02361-x.

Additional file 1: Supplementary Figure 1. B3GNT6 mRNA levels in healthy tissues (green) compared with cancer tissues (red), downloaded from IST online database (http://ist.medisapiens.com/).

Additional file 2: Supplementary Figure 2. The heat map shows patients with high B3GNT6 level (above) and low B3GNT6 level (below) and their gene expression in proteasome pathway.

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Authors’ contributions
CY and SHK: design of the work, acquisition, analysis, interpretation of the data, software, and draft manuscript writing. YZ: the acquisition, analysis, interpretation of the data, and software. CL: conception, design of the work, data, software, and draft manuscript writing. CY and SHX: design of the work, acquisition, analysis, interpretation of the data, and manuscript revision. The author(s) read and approved the final manuscript.

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Availability of data and materials
The datasets generated and/or analyzed during the current study are available in the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/), TCGA dataset (https://www.portal.gdc.cancer.gov/), MediSapiens IST Online database (http://ist.medisapiens.com/), and the Human Protein Atlas (http://www.proteinatlas.org/).

Declarations
Ethics approval and consent to participate
This study was verified and ethically approved by the Medical Ethics Committee of Xiangya Hospital of Central South University (approval number 20211223). Written informed consent was obtained from all participants for the use of surgical specimens for pathological examination. All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1 Department of General Surgery, Xiangya Hospital of Central South University, Changsha 410000, Hunan Province, China. 2 Hunan Key Laboratory of Precise Diagnosis and Treatment of Gastrointestinal Tumor, Xiangya Hospital of Central South University, Changsha 410000, Hunan Province, China. 3 International Joint Research Center of Minimally Invasive Endoscopic Technology Equipment & Standardization, Xiangya Hospital of Central South University, Changsha 410000, Hunan Province, China. 4 Department of Colorectal Surgery, 1st Affiliated Hospital of Zhejiang University, Hangzhou 310000, Zhejiang Province, China.

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