

Expression and significance of glucosylceramide synthase in colorectal carcinoma tissues

C. WANG, J.N. LIU, L. XU, Y.L. MU, P. SUN

Department of Oncology, Yantai Yuhuangding Hospital, Affiliated to Qingdao University, Yantai, Shandong, China

Abstract. – **OBJECTIVE:** To investigate the expression of glucosylceramide synthase (GCS) in colorectal carcinoma tissues.

MATERIALS AND METHODS: Immunohistochemical method was used to detect the expression of GCS in 188 cases of colorectal carcinoma tissues and 80 cases of non-cancerous colorectal tissues. The expression of GCS and the relation with age, gender, tumor location, Tumor stage, differentiation, lymph node metastasis were analyzed. Thus, we further discussed whether to accept neoadjuvant chemotherapy can affect the expression of GCS.

RESULTS: GCS was overexpressed in colorectal carcinoma tissues compared with non-cancerous colorectal tissues; the expression of GCS was associated with lymph node metastasis, but not associated with age, gender, tumor location, differentiation and Tumor stage. What's more, the positive expression rate of GCS in patients who receiving neoadjuvant chemotherapy was higher than that in untreated patients.

CONCLUSIONS: GCS was upregulated in colorectal carcinoma tissues. It might not only play an important role in controlling the generation and progression of colorectal carcinoma, but also be a factor of MDR.

Key Words:

Colorectal carcinoma, Glucosylceramide synthase, Multidrug resistance.

Introduction

Colorectal cancer is one of the common malignant tumors which are threatening human's health, its morbidity and mortality are rising year by year. According to the related researches¹, about 1,200,000 people increase in colorectal cancer each year, of them, 600,000 died of this disease. Surgery is still the main treatment for early colorectal cancer, and the post-operative five-year survival rate can be as high as 90%^[2]. But for patients with advanced, surgery alone does not improve the prognosis. Chemotherapy

has become an important method for post-operative and advanced colorectal cancer. However, the effect of chemotherapy for some cancer patients is not ideal, the mechanism of multidrug resistance is recognized as the direct reason of the chemotherapy failure.

Multidrug resistance (MDR) is defined as the concept that development of resistance to one drug can lead to the resistance to another drug (different structure and target)³. The history of MDR started in 1974, when Ling and Thompson⁴ described a stable colchicine-resistant cell clone derived from a CHO cell line by a single-step selection, and discovered that the resistant cells did not allow colchicine to enter the cytoplasm. It can be divided into primary and acquired resistance. Both primary and acquired resistance can be caused by alterations to drug metabolism (sequestrations or enhanced detoxification) or modifications to the drug targets^{3,5-7}.

The mechanism of tumor MDR is more complex⁸, of which mediated by glucosylceramide synthase (GCS) has gradually attracted the attention of the people in recent years. Ceramide, a lipid second messenger, induces growth arrest and/or apoptosis in cancer cells⁹. GCS as a pivotal enzyme can transfer a glucose residue from UDP-glucose to ceramide for the synthesis of glucosylceramide (GlcCer). After a series of glycosyltransferase modified, GlcCer can form new glycosphingolipids. These glycosphingolipids are closely related to the biological behavior of tumor cells, which not only help to maintain the normal cells structure and function, but also take part in cell proliferation, differentiation, and signal transduction¹⁰. Thus, GlcCer can fight drug-induced apoptosis and allow cellular escape from ceramide-induced apoptosis, which plays an important role in regulating cell proliferation and survival. Ceramide levels are decreased in cancer cells, which also can protect cancer cells from the challenges of chemotherapy, leading to multi-drug resistance.

Studies have confirmed that GCS was involved in multidrug resistance mechanism and overexpressed in a variety of malignant tumor tissues, including leukemia, thyroid and breast cancer¹¹⁻¹³. At present, the relationship of GCS and clinical significance in colorectal cancer still remain unclear. In this experiment we used immunohistochemical method to detect the expression of GCS and explored the differences of GCS expression and its clinical significance. It maybe provide the reference for the choice of chemotherapy and related prognosis.

Materials and Methods

Clinical Data

Between February 2011 and September 2013, specimens are obtained from patients with colorectal adenocarcinoma proved by pathology who underwent surgical operation in Department of Gastrointestinal Surgery, Yantai Yuhuangding Hospital. In all of the 188 cases of specimens, 122 cases were untreated before operation, while the other 66 cases received neoadjuvant chemotherapy. Among the untreated colorectal cancer tissues, 78 cases were male and 44 female,

the patients ranged in age from 41 to 77 years (mean age 59.5 years). The clinicopathological features have been summarized in Table I, such as age, gender, tumor location, Tumor stage, differentiation, lymph node metastasis. Meanwhile, 80 cases of non-cancerous colorectal tissues were served as control group. 44 cases of the colorectal carcinoma patients who were untreated before operation received clinical followup at a median of 29 months (range, 15-36 months).

The use of these tissues was approved by the Research Ethics Committee of Qingdao Medical University and we obtained written informed consent from all participants involved in our study.

Immunohistochemistry Procedure and Evaluation

Specimens were fixed with 10% formaldehyde, paraffin embedded. And tissue blocks were cut into 4- μ m-thick sections, deparaffinized, and rehydrated. For immunostaining, antigens were retrieved in a steaming sodium citrate buffer (10 mmol/L sodium citrate, 0.05% Tween 20, pH 6.0) for 20 minutes. Next, after blocking with a 3% H₂O₂-methanol solution, the slides were incubated with primary antibody against GCS

Table I. GCS expression and clinicopathological characteristics of colorectal cancer

Variables	n	GCS		Positive rate (%)	χ^2	p-values
		-	+ ~ + + +			
Gender						
Male	78	38	40	51.28	0.12	p > 0.05
Female	44	20	24	54.55		
Age (years)						
≥ 60	50	20	30	60.00	1.93	p > 0.05
<60	72	38	34	47.22		
Differentiation						
Low	35	17	18	51.43	0.13	p > 0.05
Moderate	60	29	31	51.67		
High	27	12	15	55.56		
Tumor location						
Rectum	50	25	25	50.00	0.21	p > 0.05
Colon	72	33	39	54.17		
Tumor stage						
T1-2	39	19	20	51.28	3.19	p > 0.05
T3	49	27	22	44.90		
T4	34	12	22	64.71		
Lymph node metastasis						
Yes	68	25	41	60.29	4.55	p < 0.05
No	54	31	23	42.59		

(1:200), overnight at 4°C. The slides were then incubated with a horseradish peroxidase-labeled polymer for 20 minutes. At last, the slides were coloured by diaminobenzidine (DAB), counterstained with hematoxylin, dehydrated in graded ethanol, cleared in xylene and mounted. In addition, the positive control used positive sections expressing GCS. As a negative control, the sections were treated with phosphate buffered saline (PBS) instead of the primary antibodies.

Two pathologists who work in Department of Pathology, Yantai Yuhuangding Hospital evaluated all of the tumor slides stained with DAB and hematoxylin for the following morphological features and the histological tumor type according to WHO 2003 classification. GCS is mainly expressed in the cytoplasm. The score standard was evaluated by a method that combined staining intensity and percentage of positive cells. The staining intensity was scored as 0, negative; 1, light yellow; 2, brown yellow; 3, brown. The percentage of positive cells was scored as 0, <10%; 1, 10%-25%; 2, 26%-50%; 3, 51%-75%; 4, 76%-100%. The final score was calculated by multiplying the intensity score by the score for the percentage of positive cells, and it ranged from 0 to 12. A total score was considered as (-), 0-3; (+), 4-6; (++) , 7-9; (+++) , 10-12. For GCS, cytoplasmic staining was considered positive, with a score ≥ 3 defined as high expression and < 3 , as low expression.

Statistical Analysis

All data represent the mean \pm SD. Student's *t*-test and χ^2 -test were used to calculate statistical significance using the SPSS 19.0 (SPSS Inc.,

Chicago, IL, USA). Statistical significance was defined as $p < 0.05$.

Results

GCS Expression in Colorectal Cancer Tissues and Non-Cancerous Colorectal Tissues

Immunohistochemical method was used to detect the expression of GCS in 122 cases of colorectal carcinoma tissues (untreated group before operation) and 80 cases of non-cancerous colorectal tissues. The positive rate of GCS in colorectal carcinoma tissues and non-cancerous colorectal tissues were 52.46% (64/122) and 36.25% (29/80). GCS was overexpressed in colorectal carcinoma tissues compared with non-cancerous colorectal tissues ($p < 0.05$ Figure 1). Furthermore, the positive rate of GCS in colorectal cancer patients who receiving neoadjuvant chemotherapy were 68.18% (45/66). Its GCS expression also higher than that in non-cancerous colorectal tissues.

Relationship Between GCS Expression and Clinicopathological Characteristics of Colorectal Cancer (Table I)

There was no statistical significance in the relationship between GCS expression and clinicopathological characteristics of colorectal cancer, including gender, age, differentiation, tumor location and tumor stage. But the frequency of lymph node metastasis was significantly higher in the high GCS expression group than those in the low GCS expression (Table I).

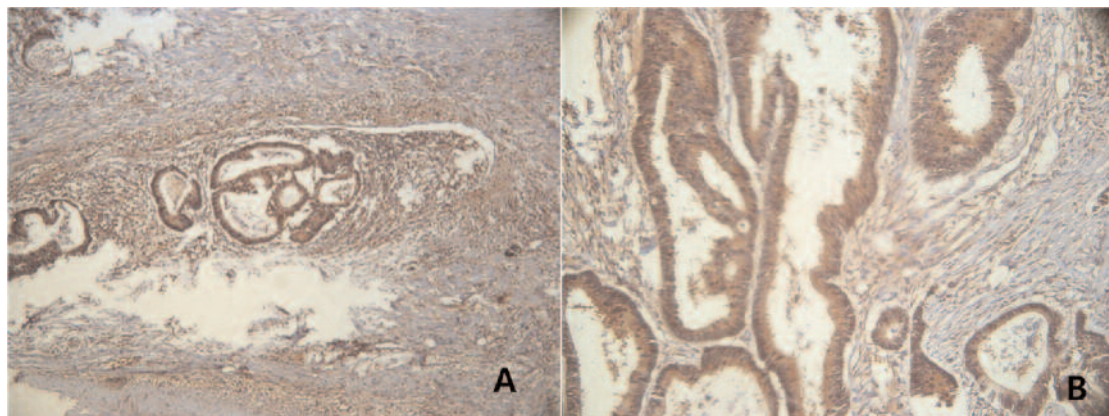


Figure 1. Expression of GCS protein in colorectal cancer tissue.

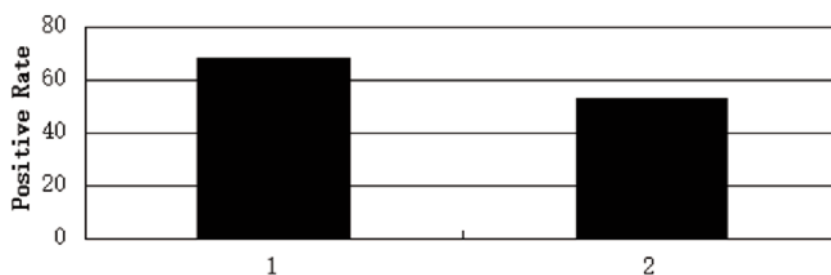


Figure 2. Correlation between GCS expression and neoadjuvant chemotherapy. 1. Positive rate of GCS in colorectal cancer group who receiving neoadjuvant chemotherapy before operation. 2. Positive rate of GCS in untreated group before operation.

Relationship Between GCS Expression and Neoadjuvant Chemotherapy

The positive rate of GCS in colorectal cancer patients who receiving neoadjuvant chemotherapy and untreated group before operation were 68.18% (45/66) and 52.46% (64/122). The former was significantly higher than the latter ($p < 0.05$ Figure 2).

Correlation Between the GCS Expression and the Survival

50 cases of the colorectal carcinoma patients who were untreated before operation received clinical followup. There was no statistical significance in the relationship between GCS expression and overall survival ($p = 0.982$, Figure 3).

Discussion

GCS, the key enzyme in GC metabolism, catalyzes the first glycosylation step in glycosphingolipid biosynthesis. It can transfer a glucose residue from UDP-glucose to ceramide to synthesize GC, which may downregulate the level of intracellular ceramide and inhibit apoptosis caused by ceramide. So inhibition by some methods depresses GCS expression and reverses the MDR phenotype, suggesting that GCS is of great importance in the regulation of cell apoptosis and a promising target for MDR reversal^{14,15}.

GCS is widely expressed in the eukaryotic cell membranes, especially mainly occurs on the surface of the Golgi¹⁶. The primary structure of GCS

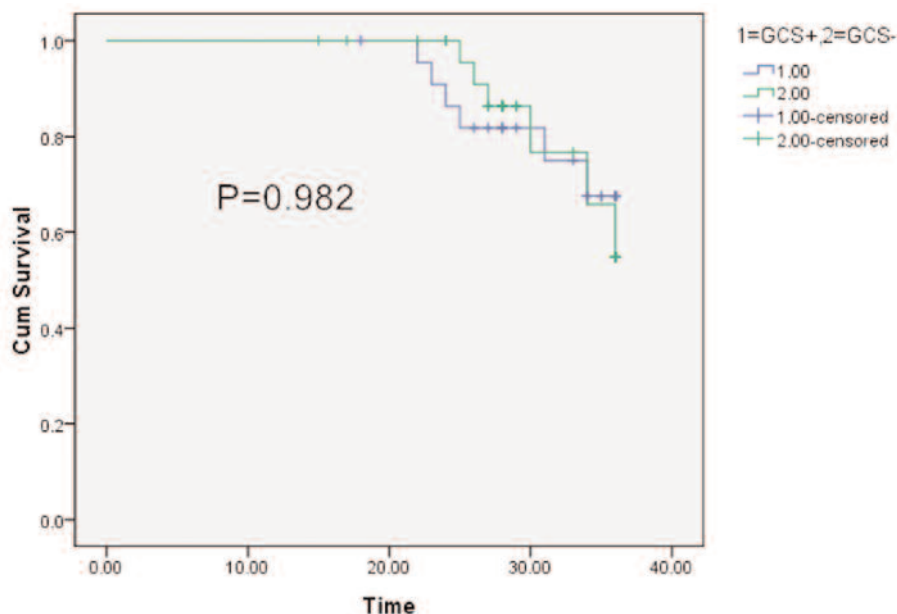


Figure 3. Overall survival.

is made of 394 amino acid residues, the end of N is signal anchor zone, the end of C is enzyme catalytic domain^[17]. Its antigenic determinant is embedded in the Golgi. Difficult separation also limits the progress in the study of GCS¹⁸.

Recent studies have shown that glucosylceramide synthase (GCS) is associated with drug resistance in cancer^{4,5,6,19}. Song et al²⁰ found that the expression of GCS protein was much higher in HCT-8/VCR than that in HCT-8. Their results indicated that suppression of GCS restores the sensitivity of multidrug resistance colon cancer cells to drug treatment. However, the study of multidrug resistance is still in the initial stage, the research on GCS expression in colorectal cancer tissues has not been reported.

This experimental results showed that the positive expression of GCS was higher than that of non-cancerous colorectal tissues. 122 cases of colorectal cancer specimens were untreated before operation. It indicated that the increased expression of GCS may be one of the causes of primary drug resistance. We also examined the relationship between GCS expression and clinicopathological features. We found that the expression of GCS was associated with lymph node metastasis, but not associated with gender, degree of differentiation and depth of invasion. This result is not consistent with previously reported data from studies on breast²¹ and papillary thyroid tumors⁵. Considering the small number of samples and differences in the study populations, results may be limited. We also found that GCS expression in colorectal cancer patients who receiving neoadjuvant chemotherapy was significantly higher than that in the untreated group. So we could consider that chemotherapy drugs may induce the high expression of GCS and increase the risk of MDR, leading to the failure of chemotherapy. And there was no statistical significance in the relationship between GCS expression and overall survival in our case load.

Conclusions

Our study demonstrate that GCS was upregulated in colorectal carcinoma tissues. It might not only play an important role in controlling the generation and progression of colorectal carcinoma, but also be a factor of MDR. What's more, inhibition of GCS activity using the corresponding method will be one of the effective ways to reverse drug resistance, but there is still no relevant

clinical trials to support, GCS targeted therapy in clinical application still need reliable clinical experiments. And there was no statistical significance in the relationship between GCS expression and overall survival in our experiment. GCS as an independent factor affecting prognosis needs to be explored in more flow up studies.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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