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ORIGINAL ARTICLE

Expression of GW112 and GRIM-19 in colorectal cancer tissues

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Summary

Purpose: *To investigate the expression of GW112 and GRIM-19 in colorectal cancer tissues.*

Methods*: Immunohistochemistry and semi-quantitative PCR were used to simultaneously detect the levels of expression of GW112 and GRIM-19 in colorectal cancer tissues and normal colorectal tissues in 39 cases.*

Results: *Expression of GW112 protein and mRNA were significantly higher in colorectal cancer tissues than in normal tissues (p<0.05). Expression of GRIM-19 protein and mRNA were significantly lower in colorectal cancer tissues than in normal tissues (p<0.05). GW112 gene mRNA copy number/GAPDH gene mRNA copy number were 0.53±0.21 and 1.81±0.65 in normal colorectal tissues and colorectal cancer tissues respectively, and GRIM-19 gene mRNA copy number/GAPDH gene mRNA copy number were 1.15±0.29 and 1.74±0.0.44 in colorectal cancer tissues and normal colorectal tissues, respectively. Expression of GW112 gene mRNA was significantly higher in colorectal cancer tissues than in normal tissues (p<0.05), and expression of GRIM-19 gene mRNA was significantly lower in colorectal cancer tissues than in normal tissues (p<0.05).*

Conclusion: *High expression of GW112 in colorectal cancer tissues and reduced expression of GRIM-19 in colorectal cancer tissues may be associated with abnormal proliferation of cancer cells and are possibly one of the reasons for development of colorectal cancer, which can provide effective targets for clinical treatment of this disease.*

Key words: *apoptosis, colorectal cancer, GRIM-19, GW112, immunohistochemistry*

Introduction

One of the major reasons for tumorigenesis is incontrollable apoptosis. Recent studies have shown that OLFM4GW112/hGC-1 (olfactomedin 4, also called GW112, hGC-1) [1] and GRIM-19 (genes associated with retinoid-IFN-induced mortality-19) [2] are functional genes associated with tumor cell apoptosis, in which GW112 is an anti-apoptotic gene, while GRIM-19 has pro-apoptotic properties, and GW112 protein and GRIM-19 protein are a pair of interactive molecules and play an important role in tumor cell apoptosis.

As an important regulatory factor of apoptosis, GW112, by its overexpression, can weaken the expression and apoptosis of IFN/ATRA and hydrogen peroxide-induced apoptosis genes, thus promoting tumor growth. Some authors have measured the transcription of GW112 and GRIM-19 in 12 kinds of human cancer cell lines by semi-quantitative RT-PCR and found that GW112 and GRIM-19 have opposite functions; GRIM-19 can promote tumor cell apoptosis, and its high expression can inhibit tumor cell proliferation [3], while GW112 has anti-apoptotic effect, and its high expression in tumor cells promotes cell growth [4]. The study made by Zhang et al. [1] has also shown that GRIM-19 ability to stimulate apoptosis can be significantly reduced and blocked by GW112, confirming the interaction between

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GRIM-19 and GW112 by means of yeast two-hybrid library screening.

Currently, few studies have investigated the roles of GW112 and GRIM-19 gene expression in colorectal cancer. In this study, GW112 and GRIM-19, the two different apoptosis regulatory factors, were selected as indicators to observe their expression in human normal colorectal tissues and colorectal cancer tissues, to be used as data and evidence in colorectal cancer research.

Methods

Materials

Thirty-nine 39 specimens of colorectal cancer tissues surgically removed in our hospital were collected from May 2009 to October 2010. Each specimen was divided into two parts, one for pathological diagnosis and immunohistochemical staining and the other one for RNA extraction. The specimens were immediately stored in refrigerator at -80°C. Normal colorectal tissues were collected from the surgically removed specimens (more than 5 cm from the tumor margin), and each specimen was also divided into two parts, one for pathological diagnosis and immunohistochemical staining and the other one for RNA extraction. The specimens were immediately stored in refrigerator at -80°C. The specimens fixed with conventional 10% methanol were cut in 4μm thick serial sections, stained with hematoxylin-eosin and embedded in paraffin. The 39 specimens included 21 cases of poorly differentiated adenocarcinoma, 14 cases of moderately differentiated adenocarcinoma and 4 cases of well differentiated adenocarcinoma. The patients from which the specimens were taken were 59.1 years old on average (range 34-77) and the male to female ratio was 1.3:1.

The instruments and reagents included Taq DNA polymerase, dNTP and other PCR reagents, purchased from Takara Biotechnology Co., Ltd (Dalian, China). Reverse transcriptase was purchased from M-MLV Reverse Transcriptase, Promega Co., China. The internal control was GAPDH, and its primers were: Forward: '5-GTCAGTGGTGGACCTGACCT-3', Reverse: '5-AGGGGTCTACATGGCAACTG-3'; the GAPDH amplified fragment length was 420bp. GW112 primers were: Forward: '5-TGAGGCACCTGCTTTTTCTT-3', Reverse: '5-ACACCAGCAAGGTTTCCAAC-3'; the GW112 amplified fragment length was 489bp. GRIM-19 primers were: Forward: '5-ACCGGAAGTGTGGGATACTG-3', Reverse: '5-GAAGCATCTGCAAGGTCCTC-3'; the GRIM-19 amplified fragment length was 287bp.

Extraction of total RNA

We used TRIzol reagent (GIBCO/BRL Gaithersburg, MD, USA) as the RNA extraction reagent, which was produced by acidic phenol one-step extraction. Before RNA extraction, RNA enzyme-free treatment was needed to ensure RNA enzyme-free environment required in the experiment, including utensils and water. RNA was extracted according to the following steps: All the pestles, homogenizers and other utensils were roasted at 200°C for 4 hrs before use to remove all RNA enzymes, including ribozyme cleavage; after cooling, they were placed in liquid nitrogen for pre-cooling, and the test tissues were quickly taken out and rubbed in mortar; TRIzol reagent was added in, and a curette was used to place tissue powder into a homogenizer; homogenization lasted several minutes. A processed RNA enzyme-free centrifuge tube was used to contain the homogenized liquid, and after chloroform was added in, centrifugation at 4°C, 12000 rounds per min and layering were conducted; the upper aqueous phase layer was transferred into a processed RNA enzyme-free centrifuge tube, isopropanol was added in, and was centrifuged at 4°C, 12000 rounds per min to precipitate RNA; the precipitate was washed twice with 75% ethanol and RNA enzyme-free deionized water was used to dissolve the precipitate. All the RNAs were digested with RNA enzyme-free DNase I to eliminate genomic DNA contamination.

Synthesis of cDNA

We randomly took 1 μl of primers (50pM), i.e. 2 μg of total RNA, and calculated the consumption of DEPC water according to RNA concentration, i.e. 15 μl. We incubated it at 70°C for 3 min, immediately placed it on ice for denaturation for 5 min, adding 5 μl of 5×buffer, 1 μl of 10mM dNTP, 1 μl of M-MLV (200 U/μl) and 0.75 μl of inhibitor (TaKaRa 40 U/μl), thoroughly mixed, and preserved at 42°C for 2 hrs.

Semi-quantitative RT-PCR

PCR amplification of GW112 and GRIM-19 genes was conducted with the template of cDNA, and GAPDH was used as control. PCR conditions: pre-denaturation at 94 °C for 3 min; denaturation at 94 °C for 30 sec; annealing at 58.5 °C for 30 sec and extension at 72 °C for 40 sec, totally 35 cycles (25 cycles for GAPDH); extension at 72 °C for 5 min. PCR product was observed by 2% agarose gel electrophoresis. PCR amplification products received stripe density scanning analysis by gel analysis and imaging system (Furi Technology FR980 type, Shanghai, China).

Real time-PCR

We used BIO-RAD Company's (California,USA) CFX96 real-time fluorescence quantification PCR instrument to measure the expression of genes in colorectal cancer tissues and their corresponding para-carcinoma tissues. The reaction system was as follows: totally 20 μl, including 10 μl of SYBR Premix EX Taq, 0.4 μl of primers (10μM), 2 μl of DNA and 7.2 μl of bistilled H_2O. We thoroughly mixed the reagents by

turning upside-down and placed them in a PCR instrument after centrifugation for amplification. GAPDH was used as internal control. The experiment was repeated three times to ensure the reliability of the results. The expression of genes in colorectal cancer tissues and para-carcinoma tissues was calculated by the following method: GRIM-19 (GW112) cycle threshold (Ct) = average GRIM-19 (GW112) Ct-level GAPDH Ct, GRIM-19 (GW112) Ct = GRIM-19 (GW112) Ct cancer tissues - GRIM-19 (GW112) Ct para-carcinoma tissues. Multiple relationships of GRIM-19 (GW112) genes in colorectal cancer tissues and para-carcinoma tissues were calculated by 2-GRIM-19 (GW112) Ct.

Immunohistochemical detection of GW112 and GRIM-19 in normal colorectal tissues and colorectal cancer tissues

Immunohistochemical staining was carried out by peroxidase-labeled streptavidin-avidin staining (SP method), and the operation steps were in accordance with the staining steps contained in the instructions.

Statistics

Values were shown as mean±standard error of the mean (SEM). The significance of differences between all groups was evaluated using one-way ANOVA with a post-hoc Student-Newman-Keuls multiple comparisons test. Statistical analyses were performed using SPSS 13.0 Software (SPSS Inc, Chicago, IL). Statistical significance was set at $p<0.05$.

Results

RNA identification

Extracted RNA qualification: UV spectrophotometer was used to measure 260/280 ratio (ratio was in the range of 1.7-2.0), indicating relatively high purity; degradation was observed in MOPS formaldehyde denaturing gel, a finding that the RNA used for this study was not degraded.

Determination of GRIM-19 and GW112 mRNA in specimens

GW112 gene mRNA copy number/GAPDH gene mRNA copy number were 0.53±0.21 and 1.81±0.65 in normal colorectal tissues and colorectal cancer tissues respectively, and GRIM-19 gene mRNA copy number/GAPDH gene mRNA copy number were 1.15±0.29 and 1.74±0.0.44 in colorectal cancer tissues and normal colorectal tissues, respectively. Expression of GW112 gene mRNA was significantly higher in colorectal cancer tissues than in normal tissues ($p<0.05$), and expression of GRIM-19 gene mRNA was significantly lower in colorectal cancer tissues than in normal tissues ($p<0.05$; Figures 1 and 2).

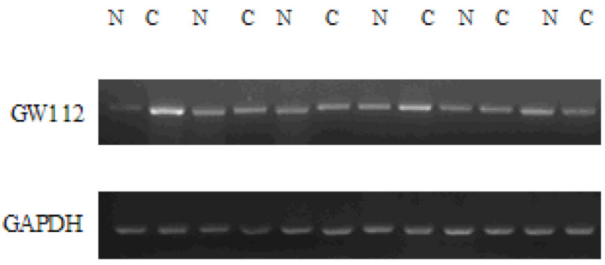

Figure 1. GW112 semi-quantitative RT-PCR strips in cancer tissues **(C)** and normal tissues **(N)**. Expression of GW112 gene mRNA was significantly higher in colorectal cancer tissues than in normal tissues (1.81±0.65 vs 0.53±0.21, $p<0.05$).

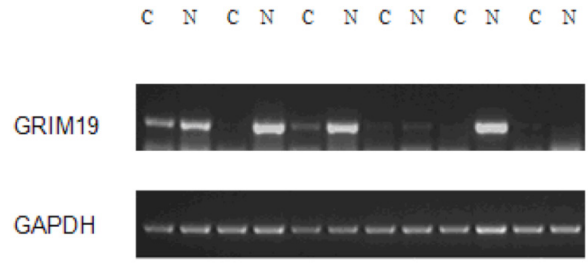

Figure 2. GRIM-19 semi-quantitative RT-PCR strips in cancer tissues **(C)** and normal tissues **(N)**. Expression of GRIM-19 gene mRNA was significantly lower in colorectal cancer tissues than in normal tissues (1.74±0.44 vs 1.15±0.29, $p<0.05$).

Expression of GW112 and GRIM-19 protein in colorectal cancer tissues and normal tissues

Expression of GRIM-19 protein in colorectal cancer tissues and normal tissues was seen in the cytoplasm and nucleus, and expression of GRIM-19 protein was significantly higher in normal tissues than in cancer tissues ($p<0.05$; Figure 3A and B).

Expression of GW112 protein in colorectal cancer tissues and normal tissues was mainly seen in the cytoplasm and cell membrane, and expression of GW112 protein was significantly higher in cancer tissues than in normal tissues ($p<0.05$; Figure 3C and D).

Discussion

Studies have found that GW112 is located in

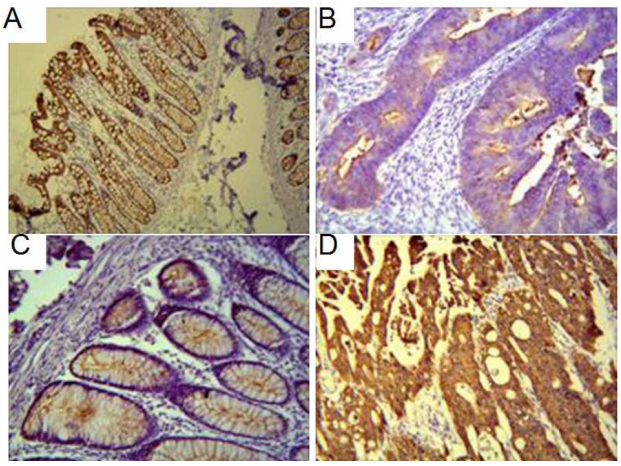

Figure 3. The expression of GRIM-19 and GW112. GRIM-19: high expression in normal tissues **(A)** and low expression in cancer tissues **(B)**; GW112: low expression in normal tissues **(C)** and high expression in cancer tissues **(D)** (x 200).

chromosome 13q14.3 and is cloned from human myeloblasts. It encodes 510 amino acids, its protein product is the precursor of olfactomedin 4, which belongs to the olfactomedin-related protein family. Some authors [1] have found that GW112 is mainly located in the mitochondria, expressed in a variety of human tumors, especially of the digestive system, and commonly seen in colorectal, pancreatic and gastric cancer tissues. Repeated experiments have shown that GW112 is indeed involved in the regulation of apoptosis, and its anti-apoptotic effect is mainly achieved by weakening H_2O2-induced apoptosis. GW112 anti-apoptotic effect should be that it can increase hypoxic, acidic and nutrient-inadequate micro-environment, thus enhancing the tumor cells to overcome some forces unfavorable for tumor cell growth, so as to promote tumor cell growth. Zhang et al. [1] have injected cells containing GW112 gene in C57/BL6 rats, and observed rapid tumor growth. Liu et al. [5] have found that GW112 product is a secretory glycoprotein that mainly adheres to cell surface, and it may bind cadherins with lectins on endogenous cell surface, thus enhancing NIH3T3 and 293T/17 cell diffusion and adhesion. Saori et al. [6], have found that GW112 is highly expressed in lung, breast and colon cancer tissues. The results of this study have also confirmed that GW112 gene is overexpressed in colorectal cancer, has enhanced invasiveness, is more easily transferred, and exerts stronger malignant biological behavior [7].

GRIM-19 is one of the recently discovered apoptosis regulatory factors and is a member of GRIM family. GRIMs are a group of recently discovered apoptosis genes involved in interferon and retinoic acid combined induction and their proteins produce apoptosis first reported by Kalvakolanu [8], who used antisense RNA knockout technology for screening and identification in breast cancer cells. Overexpression of GRIM-19 can significantly increase cell sensitivity to interferon/all-trans retinoic acid induction, thus inhibiting proliferation of tumor cells and greatly promoting apoptosis. GRIM-19 is located in chromosome 19 p13.1, its full length is 16000, it may encode a protein containing 144 amino acids, and is encoded by an open reading frame. At first, researchers believed that GRIM-19 was located in the nucleus, but further studies found that GRIM-19 was also expressed in the mitochondria, creating thus controversy. In a further study, Huang et al. [9] determined that GRIM-19 is mainly expressed in the mitochondria and is rarely expressed in the nucleus.

If expression of GRIM-19 is inhibited, tumor cell interferon/all-trans retinoic acid-induced apoptosis will be resisted. Conversely, if expression of GRIM-19 is up-regulated, cell death will be significantly increased [10]. During interferon/all-trans retinoic acid-induced tumor cell apoptosis, expression of GRIM-19 is increased in cells, a fact that promotes apoptosis.

A study has shown that STAT3 is an important oncogene [11], that can inhibit apoptosis and promote cell proliferation and is involved in genesis, development and evolution of many human malignancies [12]. It plays an important role in promoting tumor cell proliferation, inhibiting apoptosis and other activities. Abnormally activated STAT3 is present in most human tumor cells. Many studies have found that when expression of GRIM-19 is significantly reduced, expression of STAT3 will be significantly increased, indicating that reduced expression of GRIM-19 weakens inhibition of STAT3. Obviously, STAT3 activity can be obviously inhibited by GRIM-19. Gong et al. [13], reported that the genesis, development and invasiveness of colorectal cancer are related with low expression or lack of GRIM-19 in colorectal cancer tissues. Co-existence of low expression of GRIM-19 and sustained high expression of STAT3 in colorectal cancer tissues may be associated with the fact that cells are more prone to become malignant, promoting thus genesis and development of colorectal cancer.

The results of this study indicate that GW112

is highly expressed while GRIM-19 shows low expression in colorectal cancer tissues; they also confirm that GW112 inhibits GRIM-19 pro-apoptotic effect and GW112 and GRIM-19 play important roles in colorectal carcinogenesis.

In summary, expression of pro-apoptotic GRIM-19 and anti-apoptotic GW112 may be closely associated with the prognosis of patients with colorectal cancer, and may be important indicators for prognosis of such patients

References

1. Zhang X, Huang Q, Yang Z et al. GW112, a novel antiapoptotic protein that promotes tumor growth. Cancer Res 2004;64:2474-2481.
2. Angell JE, Lindner DJ, Shapiro PS. Identification of GRIM-19, a novel cell death-regulatory gene induced by the interferon-beta and retinoic acid combination,using a genetic approach. J Biol Chem 2000;43:33416-33426.
3. Huang Y, Yang M, Yang H, Zeng Z. Upregulation of the GRIM-19 gene suppresses invasion and metastasis of human gastric cancer SGC-7901 cell line. Exp Cell Res 2010;316:2061-2070.
4. Kim KK, Park KS, Song SB, Kim KE. Up regulation of GW112 Gene by NF kappaB promotes an antiapoptotic property in gastric cancer cells. Mol Carcinog 2010;49:259-270.
5. Liu W, Chen L, Zhu JC et al. The glycoprotein hGC-1 binds to cadherin and lectins. Experim Cell Res 2006;312:1785-1797.
6. Saori K, Daisuke K, Ryosuke M et al. Specific overexpression of OLFM4GW112/hGC-1 mRNA in colon, breast and lung cancer tissues detected using quantitative analysis. Cancer Sci 2007;98:315-320.
7. Li Xia, Li Yuyuan, Hu Jianguo et al. Expression of anti-apoptotic gene GW112 in gastric cancer tissues and its clinical significance. Acad J Guangzhou Med Coll 2007;35:12-15.
8. Kalvakolanu DV.The GRIMs: a new interface between cell death regulation and interferon/retinoid induced growth suppression. Cytokine Growth Factor Rev 2004;15:169-194.
9. Huang G, Lu H, HaG A et al. GRIM-19, a cell death regulatory protein, is essential for assembly and function of mitochondrial complex I. Mol Cell Bio1 2004; 24:8447-8456.
10. Lufei C, Ma J, Huang G et al. GRIM-19, a death-regulatory gene product, suppresses Stat3 activity via functional interaction. EMBO J 2003;22:1325-1335.
11. Leong PL, Andrews GA, Johnson DE et al. Targeted inhibition of STAT 3 with a decoy oligonucleotide abrogates head and neck cancer cell growth. Proc Natl Acad Sci USA 2003;100:4138-4143.
12. Xu Xueliang, Wang Hongcheng. STAT3 and tumor. J Luzhou Med Coll 2006;29:169-171.
13. Gong LB, Luo XL, Liu SY et al. Correlations of GRIM-19 and Its Target Gene Product STAT3 to Malignancy of Human Colorectal Carcinoma. Chin J Cancer 2007;26:683-687.