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The Expression of Human Tumor Suppressor Gene *beclin 1* is Down-regulated in Gastric and Colorectal Cancer*

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Abstract Human tumor suppressor gene *beclin 1* regulates cell growth through autophagy. The mRNA expression of *beclin 1* was reported to be down-regulated in breast cancer with high frequency of loss of heterozygosity (LOH). However, there was no report about the expression levels or the regulatory mechanisms of *beclin 1* in gastric and colorectal cancer. Both the mRNA and protein expression levels of *beclin 1* was detected in the tissues of gastric and colorectal cancers, as well as the aberrant DNA methylation and LOH related to the expression of *beclin 1*. By comparing with normal tissues adjacent to the tissue of these tumors, it was found that *beclin 1* mRNA expression levels were significantly decreased in gastric tumor tissue. Furtherly by explorating the 5′ region of *beclin 1* gene sequence, a large and dense CpG island was discovered and meanwhile methylations in the promoter and the intron 2 regions of *beclin 1* were found in both gastric and colorectal tumors. And LOH was found in gastric tumors. These findings suggested that aberrant DNA methylation, as well as LOH, were involved in the regulation of *beclin 1* expression in gastric and colorectal cancer.

Key words *beclin 1*, DNA methylation, LOH, gastric cancer, colorectal cancer, transcriptional regulation

Autophagy is a regulated biological process that plays an important role in balancing synthesis and degradation of cellular proteins [1]. Loss of ability in controlling autophagy appears to be associated with increased tumor incidence [2, 3]. *Beclin 1* is a tumor suppressor gene originally identified as a new Bcl-2 interacting protein by yeast two-hybrid[4]. *Beclin 1* was reported to promote autophagy in autophagy-defective yeast strains and carcinoma cells [5]. It might also functions as a pro-apoptotic molecule via enhancing caspase-9 activity in MNK 1 cells and as an anti-apoptotic molecule in HepG2 cells [6, 7]. *Beclin 1* gene maps to human chromosome 17q21, a region that shows a high frequency of allele loss in human prostate, breast and ovarian cancers [8 ~12]. *Beclin 1* encodes an evolutionarily conserved 60 ku coiled coil protein that is widely expressed in human normal adult tissues [12]. Decreased expression of *beclin 1* was detected in breast tumors and cancer cell lines[8], but its expression levels and the regulatory mechanisms in other cancers remained to be determined.

Changes in DNA methylation patterns have significant effects on human tumorigenesis. Increasing evidences indicated that hypermethylation of CpG islands in 5′ regulatory region led to transcriptional silence of many genes in cancer[13, 14]. Inspection of the 5′ region of *beclin 1* discovered a large and dense CpG island ranging from the promoter to the intron 2, which motivated us to investigate the changes of DNA methylation of *beclin 1* in cancers.

In this study, we detected the expression levels of *beclin 1* in the tissues of gastric and colorectal cancers.

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Furthermore, we investigated the effects of DNA methylation and loss of heterozygosity (LOH) on the down-regulated gene expression of *beclin 1* in these gastric and colorectal cancer tissues in order to define the possible mechanism for the effect of *beclin 1* on these cancers.

1 Materials and methods

1.1 Tissue samples

20 pairs of gastric and colorectal tumor tissues and matched adjacent normal tissues of newly diagnosed patients from the First Affiliated Hospital, China Medical University (Shenyang, China) were used in this study after Institutional Review Board approval had been done. All patients provided written informed consent. The mean age of these patients was 56.5 years-old in gastric cancer (range, 37～80) and 68 years-old in colon carcinomas (range, 51～79). Clinicopathologic parameters including gender, age, tumor size, tumor depth, tumor classification and the presence of lymphatic invasion were from clinical records. Tumors were selected solely on the basis of availability. All samples of normal tissues were derived from sites adjacent to, but at least 1 cm away from, the cancer tissues. After being microdissected with the assistance of pathologists, tissues were immediately frozen and stored in liquid nitrogen.

1.2 mRNA expression analysis

Total RNA was isolated from tissue samples by Trizol reagent kit (Invitrogen) according to the manufacturer′s protocol. Real-time quantitative RT-PCR was performed using gene specific primers and TaqMan fluorescent hybridization probes. β-Actin was amplified as internal control to normalize the quantity of specific mRNA. The primer sets for real-time PCR analysis of *beclin 1* are listed as follows: 5′ TGCA-ACCTTCCACATCT 3′ (forward), 5′ TTCCACG-GGA ACACTG 3′ (reverse), PCR product = 84 bp; 5′ AAACGGCTACCACATCCAAG 3′ (forward), 5′ AAGTGAGAATGTGGCGTTTT 3′(reverse), PCR product=430 bp; 5′ FAM-CACAGTGGACAGTTTG-GCACAATCA-TAMRA 3′ (*beclin 1* probe); 5′ FAM-CCCGACCCGGGGAGGTAGTGACG-TAMRA 3′ (β-actin probe). The authenticity of PCR products were confirmed by DNA sequencing. First strand cDNA was obtained from total RNA (0.5 μg) and oligo (dT) using the Reverse Transcription System (Promega). A total reaction volume of 20 μl contains 1× PCR buffer, 1.5 mmol/L $MgCl_2$, 200 μmol/L each of deoxynucleotide triphosphate (dNTP), 200 nmol/L of each gene specific primers, 1 U Taq DNA polymerase (Promega), 200 nmol/L Taqman probe of *beclin 1*, 150 nmol/L β-actin probe and 1 μl first- strand cDNA template. The PCR reagents were all from Promega Corporation. The PCR started at 94℃ for 3 min, then 45 cycles at 94℃ for 15 s, 60℃ for 12 s and 72℃ for 12 s. Quantitative RT-PCR was performed on a PRISM 7300 Real-Time PCR Thermal Cycler (Applied Biosystem).

1.3 Immunohistochemistry

Protein expression of *beclin 1* was performed on gastric and colorectal tissues fixed by formalin and embedded by paraffin. Deparaffinage of slides of 4 μm sections with xylene and antigen retrieval were accomplished by heat. The sections were then incubated in 3% hydrogen peroxide at room temperature for 5 min to block endogenous peroxidase activity. The slides were then incubated with rabbit anti-beclin 1 polyclonal antibody (Cell signaling, USA) at 1∶300 dilution at 4℃ overnight. Then the slides were rinsed three times in PBS for 5 min each time, following by the incubation of biotin-labeled rabbit anti-rabbit secondary antibodies for 1 h at room temperature. After washing with PBS three times, staining was performed using 3, 3′ diaminobenzidine, and sections were counterstained with hematoxylin. Positive cases were defined by the presence of cytoplasmic staining with red/brown color in at least 30% cells.

1.4 LOH analysis

Genomic DNA of tumor samples and their matched normal samples was extracted with the Dneasy Tissue Kit (Qiagen). A Taqman-based quantitative PCR method was used to detect the copy number of *beclin 1* with 18S rDNA as an internal control. Primer sets used for quantitative PCR was as follows: 5′ TCTGCCTTCCTCTGTAG 3′ (forward), 5′ TTCCACGGGAACACTG 3′, PCR product=109 bp; 5′ ACATCCAAGGAAGGCAGCAG 3′ (forward), 5′ TTCGTCACTACCTCCCCGG 3′ (reverse), PCR product = 171 bp; 5′ FAM-CACAGTGGACAGT-TTGGCACAATCA-TAMRA 3′ (*beclin 1* probe); 5′ FAM-CGCGCAAATTACCCACTCCCGA-TAMRA 3′ (18S rDNA probe). PCR products were sequenced to verify their authenticity. Each of 20 μl reaction mixture contains 2.5 mmol/L $MgCl_2$; 200 μmol/L each of dNTP; 100 nmol/L of each gene specific primers, 200 nmol/L Taqman probe, 1 U Taq DNA polymerase

(Promega) and 100 ng genomic DNA. The cycling conditions consisted of an initial incubation at 95℃ for 3 min followed by 45 cycles at 95℃ for 15 s, 60℃ for 15 s and 72℃ 30 s.

1.5 Sodium bisulfite modification and sequencing

The methylation status of *beclin 1* CpG island was determined by bisulfite sequencing as previously described[15]. 500 ng～1 μg genomic DNA was treated with sodium bisulfite modification. The *beclin 1* CpG island was divided into four regions from –528 to 977. Primers of the first region (nt –528 to –65) were 5′ TTGTTGTTGTTTTGAGATGGAGTT 3′(forward) and 5′ AAAAATATAAAAACCAAAACC 3′ (reverse) for nested PCR, and were 5′ GTTTTTTAAAG-TGTTGGAATTATAAG 3′ (forward) and 5′ AACTC-CTAATCCACAAACTCACAA 3′ (reverse) for BSP. Primers of the second region (nt -83 and 164) were 5′ AGTTTGTGGATTAGGAGTTTTTGTT 3′(forward), 5′ TAAAAATTCCCAAACTCCCTTCTA 3′(reverse) for nested PCR, and 5′ GGGTTTGTGAGTTTGTGG-ATTAG 3′ (forward) and 5′ AAAAAAAACTCCA-ATAAAAACC 3′ (reverse) for BSP. Primers of the third region (nt 137 to 400) were 5′ TTTTGGGTT-TTAAATTGTTTTTGTT 3′(forward) and 5′ TTAA-ACCCTTCCATCCCTAAAAC 3′(reverse) for nested PCR, and 5′ ATTTTAGAAGGGAGTTTGGGAATT 3′ (forward), 5′ TTAAACCCTTCCATCCCTAAAAC 3′ (reverse) for BSP. Primers of the fourth region (nt 733 to 977) were 5′ TTGTAATTTTAGTATTTTGGGA-GAT 3′(forward) and 5′ CTCTATTACCCAAAC-TAAAATACAATAATA 3′(reverse) for nested PCR, and 5′ ATATTGTGGATTTTTGAGAGTTTTT 3′ (forward) and 5′ AAATCTTTCTTTTACTACTAAA-AACTCTCT 3′ (reverse) for BSP. The first round PCR was performed in a total volume of 20 μl for each sample containing 1×PCR buffer, 1.5 mmol/L $MgCl_2$, 200 μmol/L of each dNTP, 200 nmol/L of each gene specific primers, 1 U Taq DNA polymerase (Promega) and 2 μl DNA with bisulfate modified as template. The PCR mixes were subjected to an initial incubation at 95℃ for 3 min, followed by 25 cycles of 95℃ for 30 s, and annealing at 58℃ for 30 s and 72℃ for 30 s. Final extension was done by incubation at 72℃ for 7 min. 1 μl of the first round PCR product was used as template for nested PCR. The nested PCR mix and reaction conditions were similar to the first round except for an increase up to 35 cycles. PCR products were separated and purified for ligation to TA cloning vector (Promega). Five clones of each sample were sequenced.

1.6 Statistical analysis

Data are ($\bar{x} \pm s$) deviation. Fisher's exact test or ANOVA was used to analyze associations between *beclin 1* mRNA expression and clinicopathological features. χ^2 Test was used to assess relationship between mRNA and protein expression of *beclin 1* in tumor tissues. $P < 0.05$ was considered a statistically significant difference.

2 Results

2.1 *Beclin 1* mRNA expression was down-regulated in gastric tumors

From the expression of *beclin 1* in 20 gastric cancer cases and 20 colorectal cancer cases measured by quantitative RT-PCR, the *beclin 1* mRNA level for tumor tissues was (1.65 ± 0.82) in gastric tumors and (2.28 ± 1.03) in matched normal tissues, while (1.29 ± 1.01) in colorectal tumors and (1.49 ± 1.31) in matched normal tissues, when normalized with that of β-actin (Figure 1a, b).

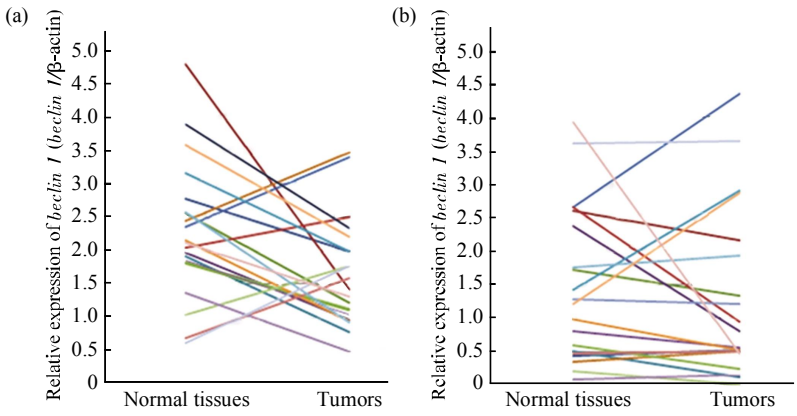

Fig. 1 *Beclin 1* mRNA expression in gastric and colorectal cancer and adjacent normal tissues

(a) Gastric cancer. (b) Colorectal cancer. Quantitative RT-PCR was carried out to detect the expression of *beclin 1* with β-actin as an internal control. 20 pairs of each cancer were analyzed, and the results were repeated at least 3 times.

t-Test analysis revealed that the mRNA expression of *beclin 1* was significantly down-regulated in gastric tumor tissues, compared to their matched normal tissues (P= 0.028, Table 1). Totally, down-regulated expression of *beclin 1* was observed in 14 out of 20 (70%) in gastric tumor tissues and 11 out of 20 (55%) in colorectal tumor tissues. Meanwhile, 7 out of 20 (35%) in gastric tumor tissues and 6 out of 20 (30%) in colorectal tumor tissues demonstrated significantly decreased profile (tumor: normal tissue < 0.5) in *beclin 1* mRNA expression.

Table 1 *Beclin 1* mRNA expression in gastric and colorectal tissues

n = 20	Gastric cancer	Colorectal cancer
Adjacent normal tissues	2.28 ± 1.03	1.49 ± 1.31
Tumors	1.65 ± 0.82	1.29 ± 1.01
	0.028	0.458

Statistical analysis was done by paired *t*-test and $P < 0.05$ was considered statistically significant.

In oder to see the relationship of *beclin 1* mRNA expression with clinicopathological phenotypes, we analyzed the relationship between *beclin 1* mRNA expression and clinicopathological parameters. We found that there were no statistically difference in the following variables, including gender, age, tumor size, tumor depth, tumor classification and the presence of lymphatic invasion, according to the *beclin 1* mRNA expression (Table 2, 3) levels.

Table 2 Realtionship of *beclin 1* mRNA expression in gastric cancer with clinicopathologic parameters

	Case number	Average of log T/N	Standard deviation of log T/N	*P*-value
Gender				
Male	13	−0.18	0.04	0.476
Female	7	−0.08	0.15	
Age (y)				
< 50	5	−0.12	0.08	0.319
⩾50	15	−0.15	0.09	
Tumor size (cm)				
< 1.5	9	−0.25	0.06	0.169
⩾1.5	11	−0.06	0.09	
Lymphatic invasion				
Positive	10	0.03	0.14	0.213
Negative	10	−0.16	0.08	
Borrmann classification				
1, 2	3	−0.31	0.01	0.243
3, 4	17	−0.06	0.12	
Depth				
Ss	12	−0.07	0.10	0.361
Se	3	−0.18	0.09	
Mp	4	−0.42	0.02	
Si	1	−0.15	—	

—: Not apply. Fisher exact *t*-test was used when number of compared group is 2 and ANOVA was performed when number of compared group is more than 3. T: Tumors; N: Adjacent normal tissues.

Table 3 Association between *beclin 1* mRNA expression in colorectal cancer and clinicopathologic parameters

	Case number	Average of log T/N	Standard deviation of log T/N	*P*-value
Gender				
Male	15	−0.18	0.20	0.596
Female	5	−0.07	0.04	
Age (y)				
< 65	9	−0.19	0.26	0.821
⩾65	11	−0.14	0.13	
Tumor size (cm)				
< 1.5	13	−0.16	0.15	0.944
⩾1.5	7	−0.15	0.19	
Lymphatic invasion				
Positive	2	0.23	0.01	0.149
Negative	18	0.16	0.16	
Dukes				
A	3	−0.20	0.14	0.462
B	10	−0.28	0.23	
C	6	0.02	0.03	
D	1	0.21	—	

—: Not apply. Fisher exact *t*-test was used when number of compared group is 2 and ANOVA was performed when number of compared group is more than 3. T: Tumors; N: Adjacet normal tissues.

2.2 Immunohistochemical analysis of beclin 1 expression of tumors

To confirm that the mRNA expression of *beclin 1* is related to its protein levels in tumors, immunohistochemical staining was performed to detect beclin 1 protein expression (Figure 2) in the recruited tissues. As shown in Figure 2, the staining intensities dramatically decreased in the gastric cancer cells (Figure 2c and 2d), compared with those in the adjacent normal tissues (Figure 2a and 2b). Similarly, the colorectal cancer cells (Figure 2g and 2h) also showed weak *beclin 1* staining, compared with those in the adjacent normal tissues (Figure 2e and 2f). The staining was cytoplasmic. Overall, negative staining was observed in 12 out of 20 (60%) in gastric tumor tissues and 10 out of 20 (50%) in colorectal tumor tissues. There was significant positive association between mRNA and protein expression of *beclin 1* in gastric and colorectal tumor tissues ($P < 0.001$) (Table 4).

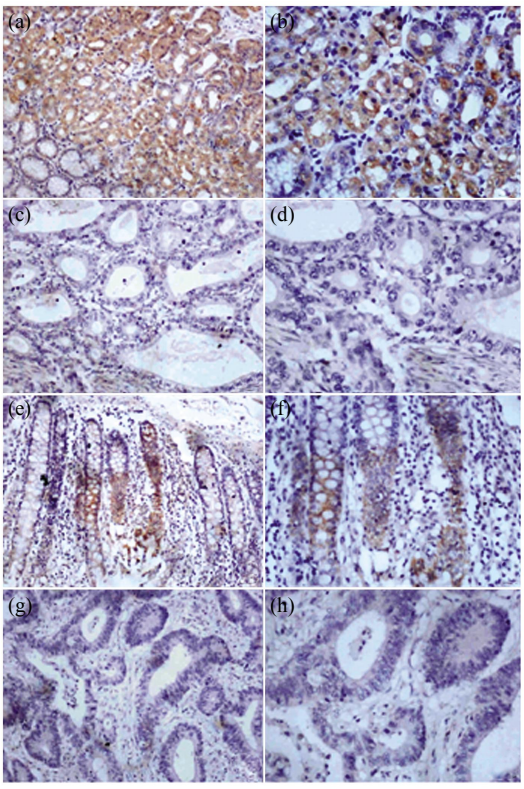

Fig. 2 Immunohistochemical detection of beclin 1 expression
(a) Low-power magnification (× 100) of gastric adjacent normal tissues. (b) High-power magnification (× 200) of gastric adjacent normal tissues. (c) Low-power magnification (× 100) of gastric tumors. (d) High-power magnification (× 200) of gastric tumors. (e) Low-power magnification (× 100) of colorectal adjacent normal tissues. (f) High-power magnification (× 200) of colorectal adjacent normal tissues. (g) Low-power magnification (× 100) of colorectal tumors. (h) High-power magnification (× 200) of colorectal tumors.

Table 4 Comparison between *beclin 1* mRNA and protein expression in gastric and colorectal tumors

Beclin 1 mRNA expression (Quantitative RT-PCR)	Beclin 1 protein expression (IHC)	
	Positive	Negative
Gastric tumors (n = 20)		
Positive (6)	6	0
Negative (14)	2	12
	$P < 0.001$	
Colorectal tumors (n = 20)		
Positive (9)	9	0
Negative (11)	1	10
	$P < 0.001$	

The relationship between *beclin 1* mRNA and protein expression was analyzed using the χ^2 test. $P < 0.05$ was considered statistically significant.

2.3 *Beclin 1* gene was mono-allelically deleted in gastric tumors

The frequent occurrence of LOH was reported to be responsible for cancers because it was often found in some gene regions of cancers. In previous study decreased mRNA expression level of *beclin 1* appeared in breast tumors [8]. In order to define the possible molecular mechanism of the low expression levels of *beclin 1* in gastric and colorectal tumors, here we studied whether the decreased expression levels in gastric and colorectal tumors were also due to LOH. As a result, there was a decrease in *beclin 1* gene copy number (tumor/normal < 1.0) in 4 out of 20 (20%) in gastric tumor tissues. Different from the observation of the gastric tumor tissues, no deletion was detected in all tumor samples of colorectal tumor tissue samples (Table 5). The tumors (T3, T4, T9 and T12) with LOH showed down-regulation of *beclin 1* mRNA expression.

2.4 Aberrant DNA methylation in the promoter and the intron 2 of the *beclin 1* gene

As hypermethylation of CpG islands in 5' regulatory region led to transcriptional silence of many genes in cancers, we inspected 5' region of *beclin 1* and a large and dense CpG island ranging from the promoter to the intron 2 (Figure 3a) was found. Therefore, we assumed that down-regulation of *beclin 1* expression may be partly due to an epigenetic modification in gastric and colorectal cancer. We subsequently detected the methylation status in this region by bisulfite sequencing. As shown in Figure 3, 6 out of 7 gastric tumors and 4 out of 6 colorectal tumors, which showed

Table 5 Beclin 1 mRNA, protein expression and LOH analysis in gastric and colorectal cancer

Gastric cancer	mRNA expression	Protein expression	LOH (Q-PCR)	Colorectal cancer	mRNA expression	Protein expression	LOH (Q-PCR)
1	–	+	–	1	–	–	–
2	–	–	–	2	+	+	–
3	–	–	+	3	+	+	–
4	–	–	+	4	–	–	–
5	–	–	–	5	–	–	–
6	+	+	–	6	–	–	–
7	+	+	–	7	+	+	–
8	+	+	–	8	–	–	–
9	–	–	+	9	–	+	–
10	–	–	–	10	–	–	–
11	–	–	–	11	+	+	–
12	–	–	+	12	–	–	–
13	–	–	–	13	+	+	–
14	+	+	–	14	–	–	–
15	+	+	–	15	–	–	–
16	–	–	–	16	–	–	–
17	–	–	–	17	+	+	–
18	–	+	–	18	+	+	–
19	+	+	–	19	+	+	–
20	–	–	–	20	–	–	–

significant down-regulation of *beclin 1* expression, exhibited obvious DNA methylation in the promoter and the intron 2 (Figure 3). Only one gastric tumor (T12, which had LOH) and two colorectal tumors (T5, T20) had no aberrant DNA methylation. The results here indicated that DNA methylation might be involved in the decreased expression level of *beclin 1* in the gastric and colorectal tumors.

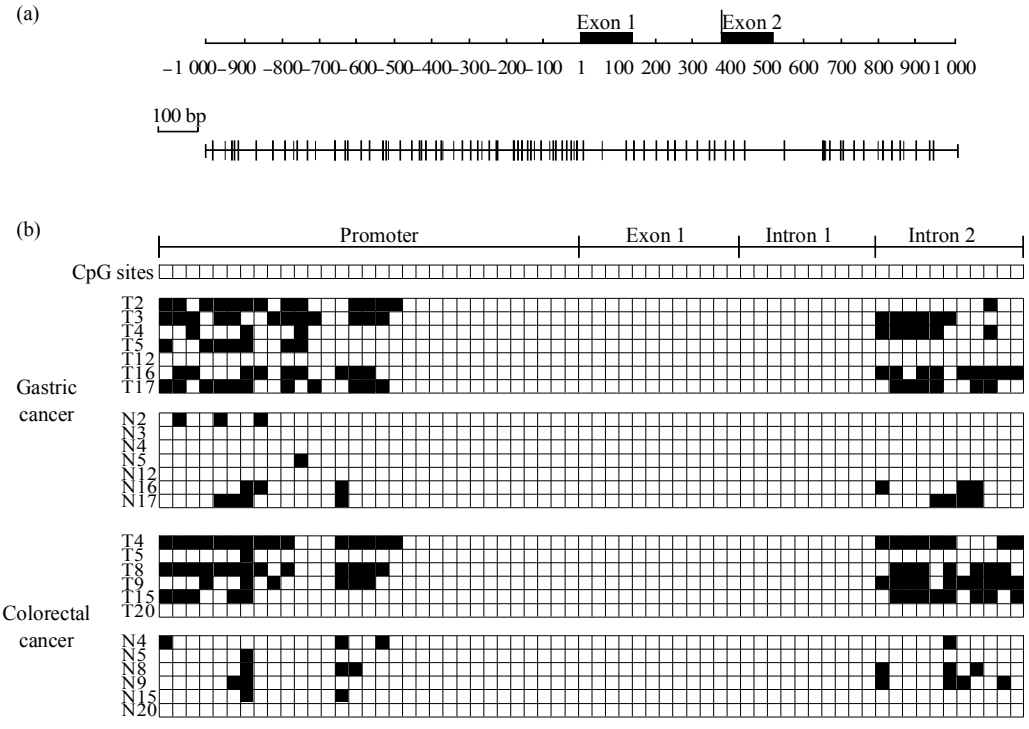

Fig. 3 Methylation patterns of *beclin 1* CpG island

(a) Genomic structure and location of 5' CpG island of *beclin 1*. (b) Bisulfite sequencing analysis of CpG islands. 7 pairs of gastric tissues and 6 pairs of colorectal tissues were subjected to bisulfite sequencing. "N" represents a normal tissue sample and "T" is a tumor sample. Black squares represent methylated CpG sites and white squares are unmethylated sites.

3 Disscussion

The purpose of this work was to investigate the express of *beclin 1* gene in some tumors and its possible molecular mechanism. In our previous study, we found that no single nucleotide polymorphism was found in *beclin 1* gene though we found that this gene was low expressed in some cancers. Hence classic genetics alone cannot explain the diversity of phenotypes in these cancers. Here we firstly wonder whether the *beclin 1* gene functions as a potential target for epigenetic modification and mono-allelic deletion in gastric and colorectal cancers. Based on the inspection of *beclin 1* genomic sequence, we have obtained the first evidence supporting this hypothesis, which was that *beclin 1* genomic sequence occupied a CpG rich (~1.5 kb), ranging from the promoter to the intron 2. This result was in agreement with the work of Weinmann *et al*.[16], which reported that the promoter-associated CpG islands of *beclin 1* were targets of E2F and contained four putative consensus Sp1 binding sites. Our methylation analysis of the *beclin 1* gene showed that the regions of the promoter and the intron 2 were methylated in both gastric and colorectal tumor tissues. The methylation of these CpG islands were consistent with the reduced *beclin 1* expression. As dense methylation was not observed in normal tissues, we deduced that this epigenetic modification was aberrant and disease-associated. In this study, we confirmed that *beclin 1* was frequently inactivated in gastric cancer, and that expression is reduced coordinately with aberrant methylation with significance in statistics. However, the expression of *beclin 1* was not reduced significantly in colorectal tumor tissues, though low level of the expression was found. The reason might be other mechanism contributing to the development of colorectal tumor in addition to the malfunction of *beclin 1* gene. Further study is needed to explore this issue. Also there will be other genetic and epigenetic reasons for this gene's silencing. This is just beginning work in the epigenetic changes of the *beclin 1* gene in gastric and colorectal cancer.

The second evidence came from the previous findings that decreased expression of tumor suppressor gene was often accompanied by allele loss in cancer[17~19], and that mono-allelic deletion of 17q21 has been found in breast, ovarian and prostate cancers with high frequencies[9~12]. As we know LOH was thought to be the most commonly genetic abnormalities for tumor suppressor genes[19, 20]. In this study, we observed that 20% of gastric tumors showed allelic deletions in gene copy number, which was significantly associated with lower mRNA expression levels, suggesting that LOH also contributed to decreased expression of this gene in gastric cancer. Taken together, the conclusion drawn from these results is that the *beclin 1* gene is a novel target for epigenetic silencing in gastric and colorectal cancer, and it also undergoes LOH in gastric cancer.

In fact, the regulation of *beclin 1* expression was a little complicated, when we went back to the gastric samples, which showed decreased expression of *beclin 1*. In some cases, T3, T5, T16 and T17, the decreased expression was due to aberrant DNA methylation. In T12, the decrease was manily from LOH; in the other cases, T2 and T4, both aberrant DNA methylation and LOH contributed to the decreased expression of *beclin 1* (Table 6). Therefore, different mechanisms might be invloved in the regulation of the expression of *beclin 1*.

Table 6 Status of LOH and aberrant DNA methylation in gastric and colorectal tumors showing *beclin 1* down-regulation

No.	LOH	Aberrant DNA methylation
Gastric tumors with *beclin 1* down-regulation		
2	−	+
3	+	+
4	+	+
5	−	+
12	+	−
16	−	+
17	−	+
Colorectal tumors with *beclin 1* down-regulation		
4	−	+
5	−	−
8	−	+
9	−	+
15	−	+
20	−	−

In a study of two colorectal tumors (T5, T20) with significantly decreased *beclin 1* expression levels, neither aberrant methylation nor LOH was found (Table 6). Then some unidentified mechanisms might be involved in the regulation of *beclin 1* expression. These mechanisms might include point mutation and bcl-2 over-expression[21]. In addition to *beclin 1* functional inactivation, a novel beclin 1 binding protein, UVRAG, might positively regulate autophagy signaling pathway mediated by *beclin 1* in colon

cancer[22]. Analysis of the regulatory mechanmisms of *beclin 1* and other contributing factors[23, 24] that affect autophagic activity might contribute to the pathogenesis of human cancers.

In conclusion, our work indicates that decreased expression of *beclin 1* is common in gastric and colorectal cancer. Aberrant DNA methylation and LOH were demonstrated to be responsible for the decrease of *beclin 1* expression in gastric cancer, and DNA methylation was also found to play a role in colorectal tumor cases. Aberrant DNA methylation and LOH of *beclin 1* associated with decreased expression suggest that epigenetic and genetic alterations of this gene may be interesting targets for cancer therapy. Futher studies on a larger population are needed to confirm the effect of LOH and DNA methylation on the transcriptional regulation of *beclin 1*.

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人类抑癌基因 *beclin 1*在胃癌和直结肠癌中表达下调的研究 *

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摘要 人类抑癌基因 *beclin 1* 通过自噬作用调节细胞生长，但在胃癌和直结肠癌中其表达水平和调控机制仍不清楚．通过检测胃癌和直结肠肿瘤组织中 *beclin 1* 基因的表达水平，及 DNA 异常甲基化和杂合子缺失对其表达的影响，发现与癌旁组织相比，35%的胃癌标本和 30%的直结肠癌标本中 *beclin 1* 基因表达显著下调．同时发现，*beclin 1* 基因 5′端存在一高密度 CpG 岛，在胃癌和直结肠癌中 *beclin 1* 的启动子区域和第二个内含子区域存在甲基化，而杂合子缺失仅在胃癌中发生．这些发现表明 *beclin 1* 基因的异常甲基化和杂合子缺失对其在胃癌和直结肠癌中的表达起调控作用.

关键词 *beclin 1*，DNA 甲基化，杂合子缺失，胃癌，直结肠癌，转录调控
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