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• GASTRIC CANCER •

Expression of MTLC gene in gastric carcinoma

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Abstract

AIM: To investigate the expression of c-myc target from laryngeal cancer cells (MTLC) gene in gastric carcinoma (GC) tissues and the effect of MTLC over-expression on gastric carcinoma cell line BGC823.

METHODS: RT-PCR was performed to determine the expression of MTLC mRNA in GC and matched control tissues. BGC823 cells were transfected with an expression vector pcDNA3.1-MTLC by liposome and screened by G418. Growth of cells expressing MTLC was observed daily by manual counting. Apoptotic cells were determined by TdT-mediated dUTP nick-end labeling (TUNEL) assay.

RESULTS: The expression of MTLC mRNAs was down-regulated in 9(60%) of 15 cases of GC tissues. The growth rates of the BGC823 cells expressing MTLC were indistinguishable from that of control cells. A marked acceleration of apoptosis was observed in MTLC-expressing cells.

CONCLUSION: MTLC was down-regulated in the majority of GC tissues and could promote apoptosis of GC cell lines, which suggests that MTLC may play an important role in the carcinogenesis of gastric carcinoma.

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INTRODUCTION

Gastric carcinoma (GC) is one of the most common malignant tumors in the world[1,2]. Numerous data have shown that some genes such as p53, c-myc, bcl-2, COX-2 and PTEN[3-6] might be associated with the gastric carcinogenesis. However, the exact molecular mechanism underlying GC remains to be fully elucidated. Therefore, it is necessary to look for novel genes to obtain a thorough understanding about gastric carcinogenesis.

c-myc target from laryngeal cancer cells (MTLC) gene, a putative target of c-myc, was recently cloned in our laboratory (GenBank access number AF527367). MTLC was located in 6q25, a chromosome region involved in various kinds of cancers[7-11]. Previous studies have shown that its protein product expressed in nuclei and might take part in the regulation of cell cycle[12], suggesting that MTLC was potentially related to the carcinogenesis. In this study, we therefore performed RT-PCR and eukaryotic transfection to reveal the relationship between MTLC and GC.

MATERIALS AND METHODS

Tissues and cell line

All the gastric cancer and matched control tissues confirmed pathologically were obtained from the First Affiliated Hospital of China Medical University. Tumor tissues were dissected from the resected specimens. The normal tissue block was taken from the distal resection margin and was apart from cancer at least 1 cm. Gastric carcinoma cell line BGC823 was kept in our laboratory.

RT-PCR

Total RNAs were extracted from cancer tissues by TRIZOL reagents (GibcoBRL, Grand Island, NY, USA), and were reverse-transcripted to the first strand of cDNA using reverse transcriptase system (Promega, Madison, WI, USA). MTLC cDNA was amplified by PCR under the following condition: first at 95 ℃for 1 min, 30 cycles at 95 ℃for 30 s, at 60 ℃for 1 min, at 72 ℃for 1.5min, and finally at 72 ℃for 10 min. PCR primers consisted of the sequences of forward: 5-ATGGATCCCTGCACTGGCTGATGAGTGTGTA-3 and reverse: 5-GTAAGCTTGAACAGTGCCTTCACCCTCG AGGT-3. β-actin gene was used as internal control.

Construction of MTLC expression vector

MTLC segment amplified by PCR was ligated to pMD-18T vector (Takara, Dalian, China) by TA cloning. The recombinant was digested by *BamH I* and *EcoR I*, and then the target fragment was recollected and cloned into pcDNA3.1 vector (Invitrogen, Carlsbad, CA, USA). Both PCR product and the expression vector pcDNA3.1-MTLC were confirmed by sequencing to avoid mutation.

Transfection and screening of BGC823 cells

BGC823 cells in logarithmic phase were seeded in 35 mm plates and cultured with DMEM containing 10 % serum overnight. Cells were transfected with 1 μg expression vector or empty parental vector by Lipofectamin 2000 (Invitrogen, Carlsbad, CA, USA) and subsequently screened by G418 at a final concentration of 5 g/L after cultured for 24 h.

Observation of cell growth

Cells transfected by pcDNA3.1-MTLC or empty parental vector were plated in 35 mm plates at a concentration of 1×10^5 cells/plate with DMEM culture containing 10 % serum. Individual plates were trypsinized daily and the total number of viable cells per plate was determined by manual counting.

Detection of apoptosis

DeadEnd™ Fluorometric TUNEL System (Promega, Madison, WI, USA) was used to determine the apoptosis of cells. 1×10^5 cells transfected by pcDNA3.1-MTLC or empty parental vector were seeded into a plate with a poly-L-lysine-coated slide on its center and grown for 24 h in DMEM culture containing 10 % serum. The cells were then maintained for additional

18 h in serum-free culture and then detected according to the protocol provided by the manufacturer. The samples were stained with propidium iodide (PI) to make a red background and then observed under fluorescence microscope.

RESULTS

Expression of MTLC mRNA in GC tissues

RT-PCR was performed in 15 paired tissues to reveal the expression levels of MTLC mRNA. The result of electrophoresis showed that the PCR product was a single band on agar gel (Figure 1). MTLC was down-regulated in cancer tissues in 9(60 %) of 15 cases after normalization by comparing the band intensities with software UVP Gelworks ID advanced version 2.5 (Figure 1).

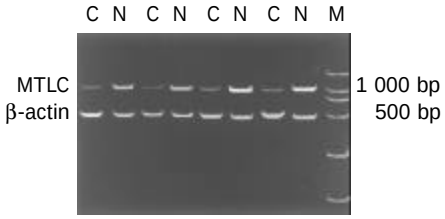

Figure 1 RT-PCR products were electrophoresed on 1 % agarose gel containing ethidium bromide. The level of β-actin was used as internal control. M: DL2000 DNA marker; C: gastric cancer tissue; N: adjacent normal gastric tissue.

Effects of MTLC expression on cell growth

One of the effects of c-myc on cells is to affect their growth properties. Therefore, we determined whether over-expression of MTLC could recapitulate this character. As seen in Figure 2, the growth rates of MTLC-expressing cells were indistinguishable from those of control cells transfected with the empty parental vector.

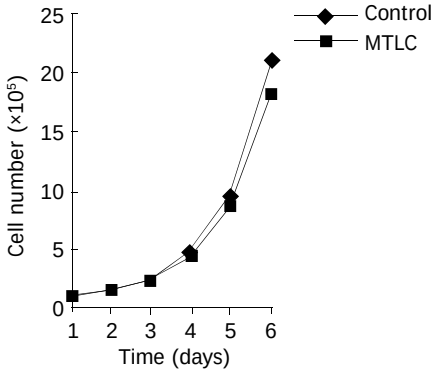

Figure 2 Cells were determined by manual counting daily. The data was analyzed by Microsoft Excel.

Promotion of apoptosis by MTLC

We studied the response of MTLC-expression BGC823 cell line to avoid its growth factors. Compared with the control

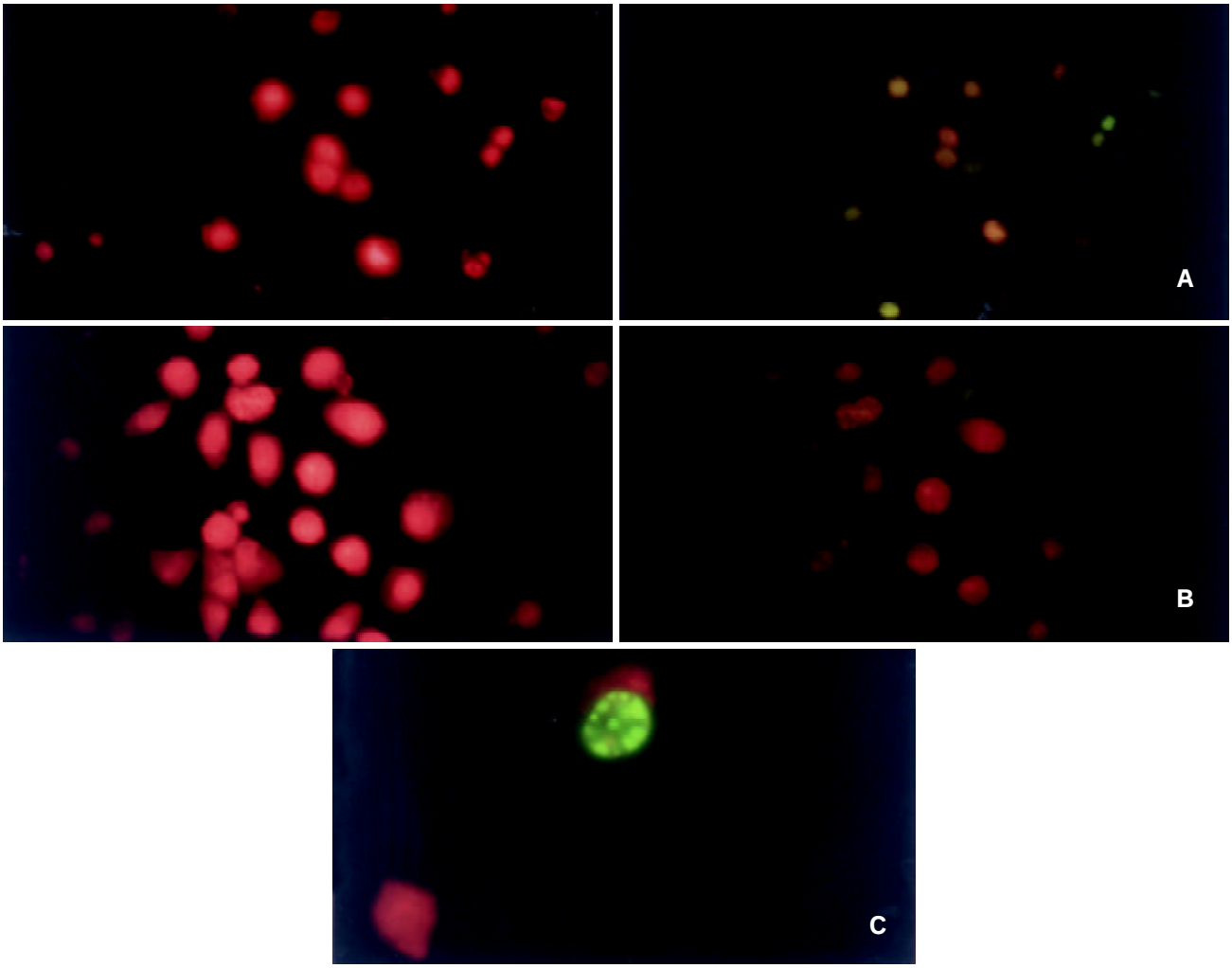

Figure 3 Apoptosis of cells were detected by TUNEL (TdT-mediated dUTP Nick-End Labeling) assay. Green fluorescence of fluorescein-12-dUTP was detected in apoptotic cells, whereas red fluorescence of PI was observed in all cells. Both signals in a same field were photographed respectively. A: MTLC-expressing cells (×400); B: control cells (×400). C: a single apoptotic cell (×1000).

cells, MTLC-expression cells showed a marked acceleration of apoptosis (Figure 3).

Table 1 Relative expressions of MTLC / β-actin in GC tissues and control tissues

Case No.	GC	Control	Ratio[a]
1	0.27	2.56	0.10
2	0.16	3.78	0.04
3	0.56	2.64	0.21
4	0.93	1.56	0.59
5	1.25	1.17	1.06
6	0.52	2.31	0.22
7	0.35	1.75	0.20
8	0.35	1.21	0.29
9	0.64	0.73	0.87
10	1.21	0.98	1.29
11	0.39	2.35	0.16
12	0.57	1.54	0.37
13	0.47	1.56	0.30
14	0.69	0.76	0.90
15	0.83	0.93	0.89

[a]The ratios less than 0.5 were defined as down-regulation.

DISCUSSION

MTLC is a novel gene cloned in our laboratory recently and has no known function. Previous studies showed that it was located in 6q25, a chromosome region involved in a variety of human malignancies, including gastric cancer. Analysis of the 5' flanking sequence also demonstrated two E-boxes on the promoter region of MTLC, suggesting that MTLC may be a target of oncogene c-myc. C-myc, a helix-loop-helix leucine zipper transcription factor, can exert considerable control over transformation, differentiation, apoptosis, and cell cycle progression through a number of target genes, including CAD[13], ODC[14,15], LDH-A[16,17], cyclin E[18], MrDb[19], telomerase/hTERT[20-25], rcl[26], IRP2[27], cdc25A[28], and JPO1[29]. It was also shown that c-myc could contribute to gastric carcinogenesis[30-36], but the exact mechanism is still not clear.

In this study, therefore, we detected the expression of MTLC mRNA in gastric cancer tissues and the effects of MTLC over-expression in gastric carcinoma cell line BGC823 to reveal the relationship between MTLC and gastric cancer. Results of RT-PCR showed that MTLC was down-regulated in 60 % (9/15) cases of gastric cancer tissues, a considerable frequency approximating to other genes suppressed in gastric cancer[37-40], suggesting that MTLC may play an important role in carcinogenesis. Furthermore, we performed gene transfection to reveal the possible function of MTLC in gastric carcinogenesis. MTLC did not affect cell growth but remarkably promoted apoptosis in response to growth factor deprivation, although we could not yet explain how this occurred. Over-expression of some other c-myc targets such as p21[41-45] and GADD45[46-51] has been reported to exhibit similar effects through p53 pathway. However, the mechanism of MTLC promoting GC cells apoptosis needs to be further studied. It is also necessary to verify the down-regulation of MTLC in GC tissues by detecting more samples with various types or clinical stages.

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