

Expression and Clinical Significance of Cytoskeleton Microtubule-Associated-Tau in Gastric Carcinoma

Chun-Hui Li¹, Meiling Hao¹, Rajina Sahi²

¹Affiliated Hospital of Chengde Medical College, Chengde 067000, Hebei Province, China

²BP Koirala Memorial Cancer Hospital

Correspondence

Chun-Hui Li

Department of Pathology
Affiliated Hospital of Chengde
Medical College
Chengde 067000
Hebei Province, China
Tel: 0086-15633142839
E-mail: Chli612@126.com

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ABSTRACT

Background and Objectives: Microtubules, the main components of spindles in the mitotic phase, can provide the suitable conditions for unlimited proliferation of tumor cells. Cytoskeleton microtubule-associated-Tau accelerates the progress of malignant tumor through microtubules. Currently, the expression of Cytoskeleton microtubule-associated-Tau in gastric cancer and the relationship between clinical pathological factors research has not yet been reported, so the report aims to elucidate the features of Cytoskeleton microtubule-associated-Tau expression in normal gastric tissue and gastric carcinoma tissue. **Materials and Methods:** The expressions of Cytoskeleton microtubule-associated protein-Tau protein and Cytoskeleton microtubule-associated protein-Tau mRNA were investigated in 60 cases of gastric carcinoma (cases) and 10 cases of normal gastric tissues (controls). Immunohistochemistry and RT-PCR were respectively used to detect the expression of Cytoskeleton microtubule-associated protein-Tau protein and Tau mRNA in the normal and carcinomatous gastric tissues. **Results:** The expressions of both cytoskeleton microtubule-associated protein-Tau protein and Tau mRNA in the carcinomatous gastric tissue were higher than in the normal gastric tissue ($P<0.05$). Likewise, both biomarkers were expressed significantly lower in the well-differentiated and moderately-differentiated gastric carcinoma than in the poorly-differentiated one ($P<0.05$). Moreover, their expressions in the gastric carcinoma without lymph node metastasis was lower than in the gastric carcinoma with lymph node metastasis ($P<0.05$). **Conclusion:** The expressions of Cytoskeleton microtubule-associated-protein, Tau-protein and Cytoskeleton microtubule-associated-protein, Tau-mRNA were significantly different between the normal gastric tissue and the gastric carcinoma tissue, and were correlated with the degree differentiation and lymph node status.

Key words: Gastric carcinoma, Cytoskeleton microtubule-associated-protein, Tau-protein, Reverse transcription polymerase chain reaction, immunohistochemistry

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INTRODUCTION

A microtubule-associated protein (MAP) is a non-microtubule structure protein combined with microtubules. To date, identified and purified MAPs include MAP-1, MAP-2, MAP-4, and the Cytoskeleton microtubule - associated protein -Tau protein.^{1,2} These proteins contribute to the assembly of microtubules, maintain the stability of microtubules, connect microtubules with other skeleton fibers, and play a wide range of other

functional roles. Cytoskeleton microtubule - associated protein -Tau proteins are the combination of microtubule polymerization proteins and microtubules, and can induce microtubule fasciculation and reinforce the stability of microtubules.³ Microtubules, the main components of spindles in the mitotic phase, facilitate smooth cell cycle processes and provide conditions for the endless proliferation of cancer cells. Cytoskeleton microtubule - associated protein

-Tau proteins assist in these functions, and may also accelerate the progress of malignant tumors. This research aimed to discuss the effects of Cytoskeleton microtubule - associated protein -Tau proteins and Cytoskeleton microtubule - associated protein -Tau mRNAs on the occurrence and development of gastric carcinoma tumors.

MATERIALS AND METHODS

Clinical data

From December 2008 to March 2010, 60 specimens of gastric carcinoma, which had been confirmed by histopathological diagnoses (cases), and 10 specimens of normal gastric tissue (controls) were collected from The Affiliated Hospital of Chengde Medical College. In these 60 cases, 49 cases were male and 11 were female. The ages of the patients ranged from 43 to 77, with an average of 58.3 ± 10.0 . A total of 28 cases were well differentiated, and 32 cases were poorly differentiated. Within the 60 cases, 25 were without lymph node metastasis and 35 involved lymph node metastasis.

Reverse-Transcription PCR Reagents

AMV first chain cDNA synthesis kits were purchased from the Shanghai ShengGong biological engineering technology service company.

The primers were synthesized by the Shanghai ShengGong biological engineering technology service company.

B-actin upstream primer (5' to 3'): CAT GTA CGT TGC TAT CCA GGC

B-actin downstream primer (5' to 3'): CTC CTT AAT GTC ACG CAC GAT

Cytoskeleton microtubule - associated protein - Tau upstream primer (5' to 3'): GTG ACC CAA GAG CCT GAA AGT

Cytoskeleton microtubule - associated protein - Tau downstream primer (5' to 3'): GGT GCA GGT CTC CTA GAA GC

50 bp DNA Ladder was purchased from Beijing Tiangen Biochemical, LTD (MD108). TRIZOL was purchased from Invitrogen; ethidium bromide was purchased from the Promeda (USA).

An amount of 1 ml Trizol extract was added to the 50-100 mg tissue samples and fully homogenated at room temperature. It was then centrifuged, and the RNA samples were extracted from supernatant and

synthesized cDNA according to the kit instructions. The amplification condition of PCR involved the following conditions: pre-degenerated at 95°C for 5 min; degenerated at 95°C for 45s, annealed at 69°C for 45s, extended at 72°C for 30s, circulated 40 times; and finally, the reactions were extended at 72°C for 10 min. The products were placed at 4°C for electrophoresis detection or placed at -20°C for long-term preservation. An amount of 10 μ l PCR product was analysed with 1.5% agarose gel electrophoresis, and after EB staining, pictures were taken using a UV fluorescence digital formatter and optical density was analysed quantitatively. The ratio of cumulative optical density of the Cytoskeleton microtubule - associated protein -Tau protein and the corresponding β -actin augmentation productstrap were considered as the relative quantity expression of the Cytoskeleton microtubule - associated protein -Tau protein.

Immunohistochemistry method

The tissue samples were fixed in 10% formalin and embedded in paraffin. Then, 4 μ m serial sections were cut. Microwave antigen retrieval was followed by immunostaining. We found that the positive signal of Cytoskeleton microtubule - associated protein -Tau protein expressed in cytoplasm of normal gastric tissue and carcinoma tissues.

Next, 5 high-power fields (magnification $\times 400$) were selected in the uniformly-stained regions. Quantitative scoring was carried out as the percentage of stained cells: $<25\%=1$ point, $25\%-50\%=2$ points, $>25\%=3$ points. Stain intensity was scored according to the following: no staining=0 point, light buff=1 point, clay bank=2 points, brown=3 points. By adding the score of stain positive cell (A) and stain intensity, the scores were assigned according to the following: negative (-): 0, weakly positive: 1-2 points, moderately positive: 3-4 points, strongly positive: 5-6 points.

Statistical analysis

Statistical analyses of the experimental data were carried out with statistical software SPSS 11.5. The values were detected using two independent sample t-tests and expressed as mean \pm standard deviation. The level of statistical significance was set at $p < 0.05$.

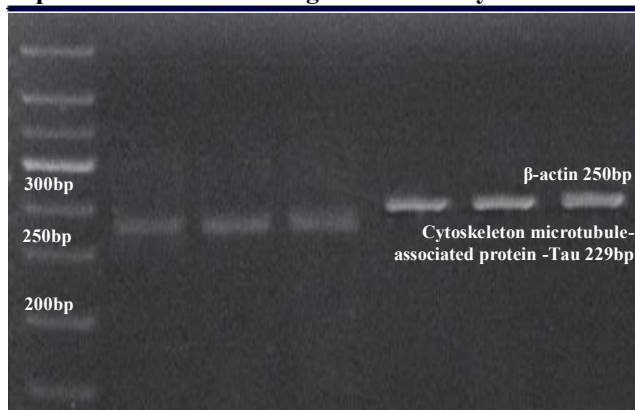


Figure 1A: Cytoskeleton microtubule-associated protein-Tau protein mRNA expression in normal gastric tissue. (x200)

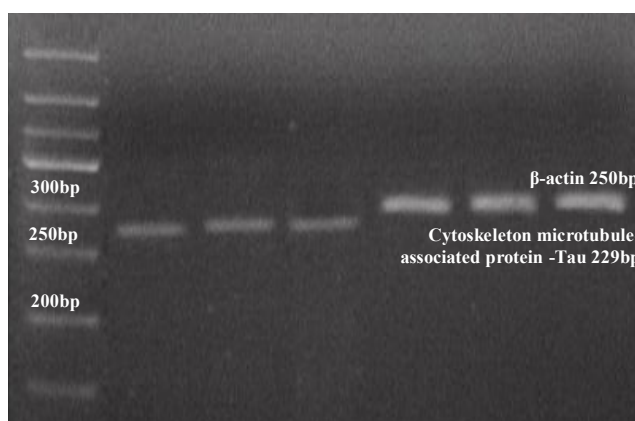


Figure 1B: Cytoskeleton microtubule-associated protein-Tau protein mRNA expression in well- and moderately-differentiated gastric carcinoma. (x200)

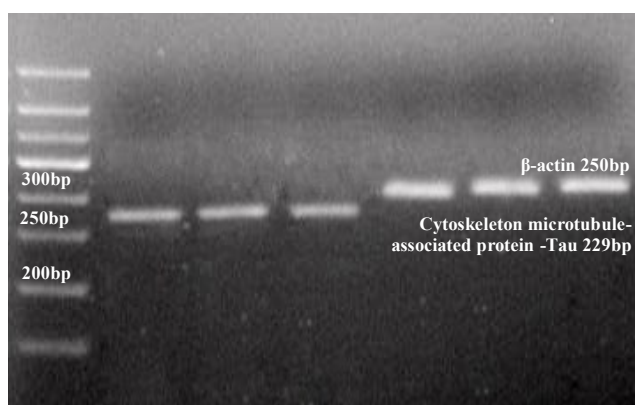


Figure 1C: Cytoskeleton microtubule-associated protein-Tau protein mRNA expression in poorly-differentiated gastric carcinoma. (x200)

RESULT

RT-PCR results

The relative amounts were found as follows: the 10 specimens of normal gastric tissue were 0.53 ± 0.01 ; the 60 specimens of carcinomatous gastric tissue

were 0.78 ± 0.08 . The well and moderately differentiated tissues were 0.70 ± 0.03 and the poorly differentiated were 0.86 ± 0.02 . The specimens without lymph node metastasis were 0.74 ± 0.07 and the specimens with lymph node metastasis were 0.81 ± 0.08 . The data was tested using two independent sample t-test. It was found that the expression of cytoskeleton microtubule-associated protein-Tau mRNA in the normal gastric tissue was lower than that in the carcinomatous gastric tissue; the expressions of Cytoskeleton microtubule-associated protein-Tau mRNA in the well- and moderately- differentiated carcinomatous gastric tissues were lower than that in the poorly differentiated carcinomatous gastric tissue; the expression of cytoskeleton microtubule-associated protein-Tau mRNA in the carcinomatous gastric tissue without lymph node metastasis was lower than that in the carcinomatous gastric tissue with lymph node metastasis. $P < 0.05$ was regarded as a significant difference. (See figures 1A, 1B and 1C and Table 1).

Immunohistochemistry results

The expression results of cytoskeleton microtubule-associated protein-Tau protein in the 10 specimens of normal gastric tissue were as follows: 3 specimens were negative, 6 specimens were weakly positive, 1 specimen was moderately positive; none of the specimens were strongly positive. The expression results of Cytoskeleton microtubule-

Table 1: The expression of Cytoskeleton microtubule-associated protein-Tau protein in normal and carcinomatous gastric tissue (mean \pm SDD)

Group	N	Mean \pm SD	P
Normal	10	0.53 ± 0.01	$P < 0.05$
Gastric Carcinoma	60	0.78 ± 0.08	
Differentiation			
Well and moderate	28	0.70 ± 0.03	$P < 0.05$
Poor	32	0.86 ± 0.02	
Lymph node metastasis			
No	25	0.74 ± 0.07	$P < 0.05$
Yes	35	0.81 ± 0.08	

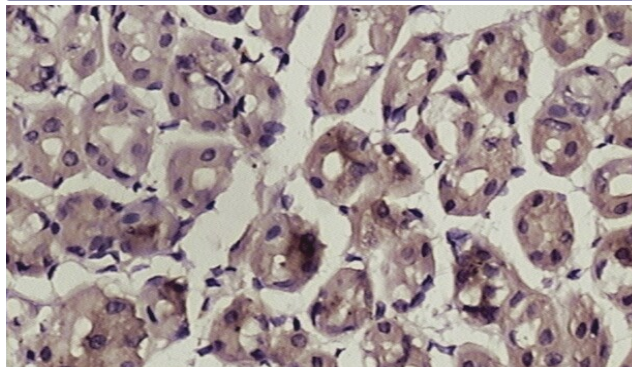


Figure 2A: Cytoskeleton microtubule-associated protein-Tau protein weak positive expression in normal gastric mucosa (magnification x200)

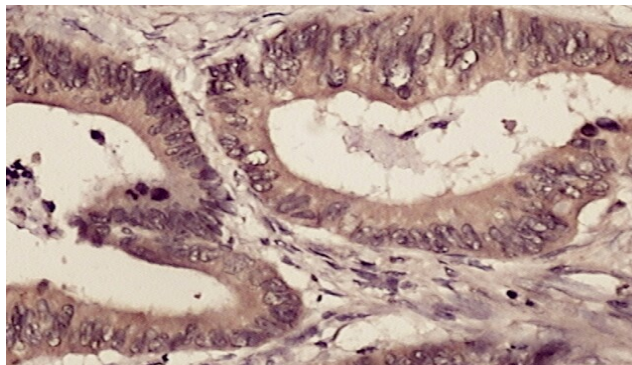


Figure 2B: Cytoskeleton microtubule-associated protein-Tau protein positive expression in moderately-differentiated gastric carcinoma (magnification x200)

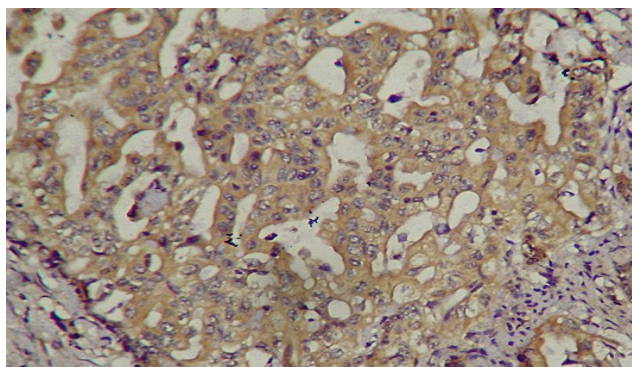


Figure 2C: Cytoskeleton microtubule-associated protein-Tau protein strongly positive expression in poorly-differentiated gastric carcinoma (magnification x200)

associated protein-Tau protein in the 60 specimens of carcinomatous gastric tissues were as follows: 10 specimens were negative, 10 specimens were weakly positive, 16 specimens were moderately positive, and 24 specimens were strongly positive. Of the 60 specimens, 28 were well differentiated and 32 were poorly differentiated. A total of 25

Table 2: The expression of Cytoskeleton microtubule-associated protein-Tau protein in normal and carcinomatous gastric tissue

Group	N	Grade				P
		-	+	++	+++	
Normal	10	3	6	1	0	<0.05
Ca	60	10	10	16	24	
Differentiation						
Well + Moderate	28	8	6	10	4	<0.05
Poor	32	2	4	6	20	
Lymph node metastasis						
No	25	6	5	12	2	<0.05
Yes	35	4	3	4	22	

specimens were without lymph node metastasis and 35 showed lymph node metastasis. The expression results of Cytoskeleton microtubule-associated protein-Tau protein in the well- and moderately-differentiated tissues were as follows: 8 specimens were negative, 6 specimens were weakly positive, 10 specimens were moderately positive, and 4 specimens were strongly positive. The expression results of Cytoskeleton microtubule-associated protein-Tau protein in the poorly-differentiated tissues were as follows: 2 specimens were negative, 4 specimens were weakly positive, 6 specimens were moderately positive and 20 were strongly positive. The expression results of Cytoskeleton microtubule-associated protein-Tau protein in the specimens without lymph node metastasis included 6 that were negative, 5 that were weakly positive, 12 that were moderately positive, and 2 that were strongly positive. The expression results of Cytoskeleton microtubule-associated protein-Tau protein in the specimens with lymph node metastasis included 4 negative, 5 weakly positive, 4 moderately positive and 22 strongly positive. The data were handled by two independent rank-sum tests. it was found that the expression of Cytoskeleton microtubule-associated protein-Tau protein in the normal gastric tissue was lower than that in the carcinomatous gastric tissue; the expression of this protein in the well- and moderately differentiated carcinomatous gastric tissues were lower than that in the poorly-

differentiated carcinomatous gastric tissue. Moreover, the expression of this protein in the carcinomatous gastric tissue without lymph node metastasis was lower than that in the carcinomatous gastric tissue with lymph node metastasis. $P < 0.05$ was regarded as a significant difference. (See Figures 2A, 2B, 2C, and Table 2).

DISCUSSION

A Cytoskeleton microtubule - associated protein - Tau protein is a phosphoprotein with a low molecular mass. Cytoskeleton microtubule - associated protein -Tau proteins are predominantly found in neuraxes and dendrites, and the adjustment of its phosphorylation level is distinct along its upgrowths. Six isomerides containing 352—441 amino acid residues can be found in human adult brains. Because of the difference between existing and three insertion elements that are lacking, the carboxyl terminus of every Cytoskeleton microtubule - associated protein -Tau protein contains 3-4 repeated sequences, which consist of 31-32 amino acid residues. Such repetitive sequences are binding sites of microtubules in normal physiological conditions. In addition, some Cytoskeleton microtubule - associated protein -Tau protein isomeride amino terminuses consist of an insert element, which contain 29 or 58 amino acid residues. Cytoskeleton microtubule - associated protein -Tau proteins degrade their dynamic instability by combining with microtubules, improve the unification velocity of microtubule protein molecules in microtubule growth ends, decrease the velocity of microtubule protein separation, and inhibit their transition to decurtation. Cytoskeleton microtubule - associated protein -Tau proteins have similar functions in vivo, and several laboratories have already transferred Cytoskeleton microtubule - associated protein -Tau genes into normal cells that do not express Cytoskeleton microtubule - associated protein -Tau proteins.^{4,5} In the study of Bonneau,⁶ In vitro, tau protein competes with taxane for controlling microtubule dynamic and loss of tau expression may render microtubules more vulnerable to the effects of taxanes. High tau protein expression was associated with better prognosis, even after adjustment for grade, hormone receptor and human

epidermal growth factor receptor-2 expression, nodal status. And in the study of Zhou,⁷ Tau expression was identified as an independent factor to predict the sensitivity of tumors to taxane-containing palliative chemotherapy in RMBC, suggesting that Tau expression in RMBC may serve as a clinical predictor for taxane-containing palliative chemotherapy. In transfected cells, Cytoskeleton microtubule - associated protein -Tau proteins enhance the stability of microtubules by combining with them,⁸ and sometimes induce microtubules fasciation. Fasciation can make microtubules stable, and Cytoskeleton microtubule - associated protein -Tau proteins may facilitate this.⁹ The main biological functions of Cytoskeleton microtubule - associated protein -Tau proteins are as follows: 1) They regulate and control the assembling of microtubules. MAPs can contribute to the formation of microtubule protein polymerization core, accelerate the polymerization of microtubule proteins, and cause the growth of microtubules by the mechanism that microtubules combining areas in peptide chain of MAP and several microtubule proteins bind simultaneously. However, MAPs can relieve the combining activity with microtubules, retard the polymerization of microtubule proteins, and delay the assembling of microtubules by the character that the overhanging function area of MAP can be phosphorylated by MAP-kinase. 2) They build, stabilize, and strengthen the cytoskeletal structure. 3) They participate in the rail directional trans-shipment of intracellular material. 4) They participate in and mediate cellular signal transduction.

Research on Cytoskeleton microtubule - associated protein -Tau proteins and Cytoskeleton microtubule - associated protein -Tau mRNAs primarily focuses on the nervous system, and rarely refers to gastric carcinoma tissue.¹⁰⁻¹² However, according to its biological functions, it can be seen that the combination of Cytoskeleton microtubule - associated protein -Tau proteins and microtubules can induce microtubules to bundle, which enhances the stability of the microtubules. As an essential component of spindles in the mitotic period, microtubules allow the cell cycle to proceed smoothly and provide conditions for infinite appreciation of cancer cells. Cytoskeleton

microtubule - associated protein -Tau proteins promote this function, which may accelerate the progress of malignant tumors.

This experiment detected the expression of Cytoskeleton microtubule - associated protein -Tau proteins and Cytoskeleton microtubule - associated protein -Tau mRNAs in both normal and carcinomatous gastric tissues. The research results indicated that the expressions of Cytoskeleton microtubule - associated protein -Tau proteins and Cytoskeleton microtubule - associated protein -Tau mRNAs in normal gastric tissue were lower than in the carcinomatous gastric tissue, and were relevant to the differentiation and lymph node metastasis of gastric carcinoma. This means that the expressions of Cytoskeleton microtubule - associated protein -Tau proteins and Cytoskeleton microtubule - associated protein -Tau mRNAs may increase in carcinomatous gastric tissue. It is thought that Cytoskeleton microtubule - associated protein -Tau proteins participate in the occurrence and development of gastric carcinoma by affecting the signal transmission of carcinomatous gastric cells. However, Cytoskeleton microtubule - associated protein -Tau proteins can also be used as the focus of targeted therapy against gastric carcinoma.

CONCLUSION

The expressions of Cytoskeleton microtubule-associated-protein, Tau-protein and Cytoskeleton microtubule-associated-protein, Tau-mRNA were significantly different between the normal gastric tissue and the gastric carcinoma tissue, and were correlated with the degree differentiation and lymph node status.

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