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Original Article

The expression of Cx43, TGFβ/Smads signaling pathways and PCNA in the occurrence and development of gastric carcinoma and the relationship among them

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Keywords:

Gastric carcinoma;
Cx43;
Smad4;
PCNA;
Signal transduction;
Gap junction;

ABSTRACT

Background: To detect the Protein expression level of Cx43, Smad4 and PCNA in normal and gastric carcinoma tissue by immunohistochemistry, western blotting and RT-PCR method.

Materials and Methods: Immunohistochemistry and western blotting were adopted to detect the expression and expression level of Cx43, Smad4 and PCNA protein in normal and carcinomatous gastric tissue. Experimental data were analyzed by combining clinical pathology data using statistical tools.

RESULTS: The expression levels of Cx43 and Smad4 in carcinomatous gastric tissue were lower than they were in normal gastric tissue. The expression level of PCNA in carcinomatous gastric tissue was higher than that in normal gastric tissue. The expression levels of Cx43, Smad4 and PCNA in groups of age and gender is not significant. The expression of Cx43, Smad4 and PCNA in different clinic pathologic features of carcinomatous gastric tissue which includes degrees of pathological differentiation, lymph node metastasis condition, depth of tumor invasion and TNM stages is statistically significant. With the progression of gastric carcinoma, the expression levels of Cx43 and Smad4 were decreased, conversely PCNA showed a high expression level.

CONCLUSION: The protein and mRNA expressions of Cx43, Smad4 and PCNA were significantly different between normal gastric tissue and gastric carcinoma and also were concerned with degree of differentiation, lymph node metastasis, TNM stages and depth of invasion, though not significant with age and gender. This suggests that they may play an important role in occurrence and development of gastric carcinoma, but also may have some interaction.

INTRODUCTION

Gastric cancer is one of the common digestive system malignant tumor with morbidity and mortality throughout the world ranked among the top of various types of tumors. Male to female ratio is about 2:1. More than 170000 deaths per year due to gastric cancer occurs in China, and accounts for about 35% of the cases in the world.[1] Many scholars believe that the occurrence and development of gastric carcinoma is a multi-factor, multi-phase, multi-step process, and in the process, the activation of oncogenes and the inactivation of tumor suppressor genes is often thought of as the basis of tumor formation and development. Adjacent cells are

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Table 1: The expression of Cx43 in normal and carcinomatous gastric tissue

Group	Case No.	Cx43		Smad4		PCNA		P
		Preserved	Reduced	Preserved	Reduce d	Preserved	Reduced	
Normal mucosa	25	23	2	21	4	12	13	P<0.05
Gastric Carcinoma	60	39	21	35	25	49	11	

□*VS*□*: P<0.05*

Table 2: The expression of Cx43, Smad4 and PCNA in different clinico pathologic features of carcinomatous gastric tissue

Group	Case No.	Cx43		Smad4		PCNA		P
		Preserved	Reduced	Preserved	Reduce d	Preserved	Reduced	
Gender								
Male	38	24	14	19	19	31	7	P>0.05
Female	22	15	7	16	6	18	4	
Age								
>60	29	21	8	18	11	24	5	P>0.05
≤60	31	18	13	17	14	25	6	
Differentiation								
Well-moderately	27	23	4	20	7	19	8	P<0.05
Poorly	33	16	17	15	18	30	3	
L/N mets								
+	35	19	16	15	20	33	2	P<0.05
-	25	20	5	20	5	16	9	
Depth of Invasion								P<0.05
Superficial muscular and submucosa	18	16	2	15	3	11	7	
Deep muscularis and serosa	42	23	19	20	22	38	4	
TNM stages								
I+II	28	23	5	21	7	19	9	P<0.05
III+IV	32	16	16	14	18	30	2	

mediated through gap junction intercellular communication which is composed of gap junction protein 43, in signal transduction processes of cell metabolism, stable internal environment, the proliferation and differentiation and other physiological Cx43 play an important role in regulating, and Cx43 sub clusters often appear on the plasma membrane forming gap junction plaque, the number directly affects the signal transmission function between two cells.[2] Smad4 is the important members of the Smads protein family and also the cell TGF-β1 / Smad signaling pathways critical components. It can almost combine with all activated R-Smads to form oligomers compounds, after which Smad4 translocation is seen to the nucleus regulate transcription of target genes. Smad4 gene mutation or deletion can lead to its functional inactivation, and thus can not play a role in regulating transcription.[3] PCNA (proliferating cell nuclear antigen) are widely expressed in S phase of cell cycle, as auxiliary of DNA polymerase δ. It plays an important role in DNA replication. It is necessary for eukaryotic cell DNA synthesis of coenzyme and its emergence is closely related to DNA synthesis in the cell cycle. It exists only in proliferation cells and tumor cells,[4] so the PCNA content is closely related to cell proliferation state, can be used as an indicator to reflect degree of tumor cell proliferation and prognosis. Cx43 plays a key role of intercellular signal transduction, as well as Smad4 which is key component of intracellular TGFβ/Smads signaling pathways may be subject to intercellular signal conduction influence, thus affecting PCNA. In development of gastric carcinoma there may be correlations among them on which the research has not been illustrated completely. By comparing the expression levels of Cx43, Smad4 and PCNA in gastric carcinoma and normal gastric mucosa tissues and their relationship with the clinical pathology characteristics (differentiation, metastasis of lymph nodes, depth of invasion, TNM stages) of gastric carcinoma, they might provide theoretical basis to the study of the pathogenesis of gastric carcinoma and new approach of clinical treatment.

Table 3: The expression of Cx43, Smad4 and PCNA Protein in normal and carcinomatous gastric tissue (±S)

Group	Case No.	Cx43	Smad4	PCNA	P
		Protein OD	Protein OD	Protein OD	
Normal mucosa	25	0.80±0.06	0.86±0.05	0.32±0.03	P<0.05
Gastric Carcinoma	60	0.37±0.06	0.36±0.07	0.76±0.06	

Table 4: The expression of Cx43,Smad4 and PCNA Protein in different clinicopathologic features of carcinomatous gastric tissue(±S)

Group	Case No.	Cx43	Smad4	PCNA	P
		Protein OD	Protein OD	Protein OD	
Gender					
male	38	0.35±0.02	0.33±0.02	0.74±0.05	P>0.05
female	22	0.39±0.03	0.41±0.03	0.79±0.04	
Age					
>60	29	0.38±0.03	0.35±0.02	0.77±0.04	P>0.05
≤60	31	0.36±0.03	0.37±0.03	0.75±0.05	
Differentiation					
Well-moderately	27	0.50±0.04	0.50±0.04	0.59±0.05	P<0.05
Poorly	33	0.23±0.03	0.25±0.03	0.90±0.08	
L/N mets					
Yes	35	0.21±0.03	0.24±0.03	0.92±0.07	P<0.05
No	25	0.59±0.04	0.53±0.04	0.54±0.04	
Depth of Invasion					
Superficial muscular and submucosa	18	0.55±0.03	0.66±0.05	0.51±0.05	P<0.05
Deep muscularis and serosa	42	0.29±0.02	0.23±0.02	0.87±0.07	
TNM stages					
I+II	28	0.51±0.03	0.49±0.04	0.62±0.04	P<0.05
III+IV	32	0.25±0.03	0.25±0.03	0.88±0.06	

MATERIALS AND METHODS

Tissue samples

We collected 60 cases of gastric carcinoma which were surgically removed and certified by pathology and were not adopted by radiotherapy and chemotherapy in Chengde Medical College affiliated hospital from August, 2011 to, October, 2012, along with 25 cases of normal gastric tissue (tissue away the tumor regarding as normal tissue for comparison). Gastric carcinoma tissues were classified according to clinico pathological parameters which included differentiation degree, lymph node metastasis, TNM stages and depth of invasion.

Immunohistochemistry staining

Hisotpathological sections (5μm) were dewaxed and rehydrated according to pathology standardized procedures, immersed in 3% H202 for 15 min at room temperature to quench endogenous peroxidase activity. After washing twice with phosphate-buffered saline (PBS) for 5 min, Sections were heated in a microwave oven at 600W for 10 minutes in 0.01M citrate buffer (pH=6.0) and unspecified binding was blocked in 5% normal rabbit serum (0.1% BSA in PBS). Sections were incubated at 37^0 C for 2 h with primary antibody, rabbit anti-rat Cx43, Smad4 and PCNA (Golden Bridge Biotechnology Co Ltd.) 1:100. Tissue sections were incubated at 37^0 C for 30 min with biotin-anti-rabbit IgG. After washing two times in PBS for 5 min, the sections were incubated with streptavidin for 30 min. Then the sections were washed two times in PBS for 5 min, and they were incubated with metal-enhanced 3,3-diaminobenzidine solution for 15 min, then they were washed two times in distilled water and counterstained with hematoxylin. In the negative control group, 5% normal rabbit serum was used in place of the primary antibody. The positive staining for Cx43 and Smad4 was expressed as brown-orange granules, which were mainly located in cell cytoplasm under microscopy, the positive staining for PCNA was expressed as red brown

Table 5: The expression of Cx43,Smad4 and PCNA mRNA in normal and carcinomatous gastric tissue (±S)

Group	Case No.	Cx43	Smad4	PCNA	P
		mRNA OD	mRNA OD	mRNA OD	
Normal mucosa	25	0.75±0.05	0.76±0.04	0.31±0.03	P<0.05
Gastric Carcinoma	60	0.37±0.07	0.30±0.07	0.69±0.08	

Table 6: The expression of Cx43,Smad4 and PCNA mRNA in different clinicopathologic features of carcinomatous gastric tissue (±S)

Group	Case No.	Cx43	Smad4	PCNA	P
		mRNA OD	mRNA OD	mRNA OD	
Gender					
Male	38	0.35±0.04	0.28±0.03	0.68±0.04	P>0.05
Female	22	0.40±0.04	0.33±0.04	0.70±0.05	
Age					
>60	29	0.38±0.05	0.31±0.05	0.70±0.05	P>0.05
≤60	31	0.36±0.04	0.29±0.04	0.67±0.04	
Differentiation					
Well-moderately	27	0.50±0.04	0.41±0.05	0.56±0.04	P<0.05
Poorly	33	0.27±0.02	0.21±0.03	0.79±0.07	
L/N mets					
Yes	35	0.26±0.03	0.20±0.03	0.83±0.06	P<0.05
No	25	0.53±0.05	0.44±0.05	0.49±0.04	
Depth of Invasion					
superficial muscular and submucosa	18	0.53±0.05	0.42±0.06	0.42±0.03	P<0.05
Deep muscularis and serosa	42	0.30±0.03	0.25±0.03	0.80±0.06	
TNM stages					P<0.05
I+II	28	0.55±0.04	0.40±0.04	0.56±0.04	
III+IV	32	0.21±0.02	0.21±0.02	0.80±0.07	

granules, which were mainly located in cell nucleus under microscopy. At least 5 high-power (x400 field) fields were chosen randomly for cell counting. The ratio of the positive distribution of Cx43, Smad4 and PCNA was calculated by dividing the number of positive cells over the total number of cells, and was expressed as percentage.

Western blotting detection

A small amount of tissue was cut with clean scissors as possible, plus 400μl single detergent lysis buffer (containing PMSF, RIPA: PMSF volume ratio of 100:1) in the homogenizer. By the BCA method the sample and the standard are added to a 96-well plate, protein concentration was determined in a microplate reader. Thirty μg tissue lysates were loaded and separated on polyacrylamide gels and then transferred to positive-charged PVDF membranes according to standard protocol. These blots were blocked for 2 h at room temperature in 5% skim milk. The target proteins were probed with primary antibodies and horseradish peroxidase-labeled secondary antibodies (Bioword Technology). β-actin was used as an indicator for equality of lane loading. Antibody positive bands were visualized using ECL Western blotting detection reagents (Solarbio Technology). The x-ray film was scanned and the band density was calculated using the Imag J software.

Preparation of RNA samples and RT-PCR

For RT-PCR experiments, 100 mg tissue (stored in liquid nitrogen) placed in the homogenizer on ice conditions was cut with ophthalmic scissors into pieces and immediately joined with precooled 1 mL Trizol reagent. RNA pellet was dried and RNA dissolved in RNase-free water was stored at -80□ for use. RNA was used as a template for amplification. Oligonucleotides as specific primers for Cx43,Smad4 and PCNA were synthesized by a company (Sangon Biotech, ShangHai, China). As a PCR control reaction β-actin was also detected in each run.

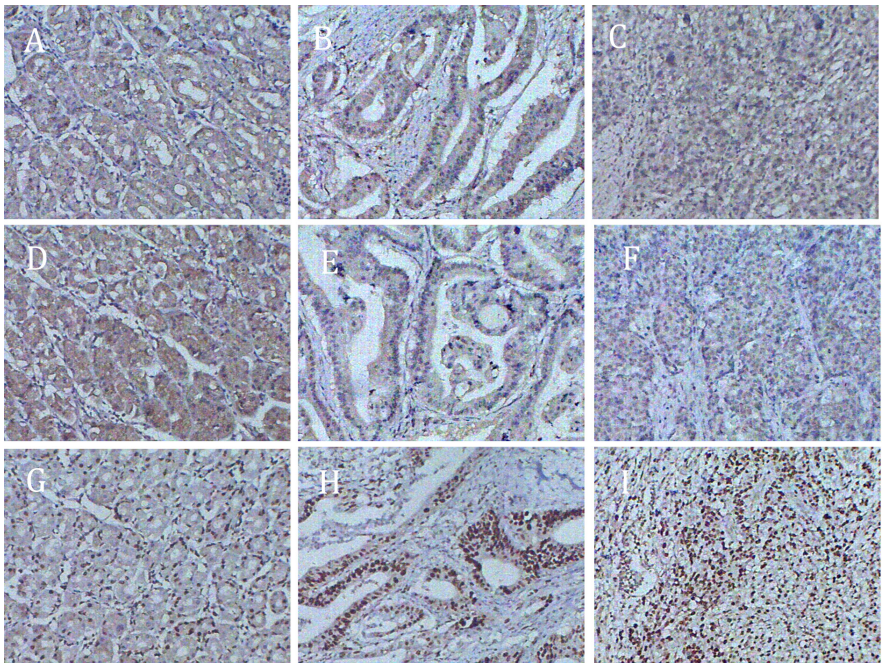

Figure 1: *A,BandC: Immunohistochemical stain for Cx43 protein (×200), A: strongly positive expression in normal gastric mucosa, B: positive expression in Well-moderately differentiated gastric adenocarcinoma, C: negative expression in poorly differentiated; D/E/F: Smad4 protein staining (×200), D: strongly positive expression in normal gastric mucosa, E: positive expression in Well to moderately differentiated gastric adenocarcinoma, F: negative expression in poorly differentiated gastric carcinoma; G/H/I: PCNA protein staining (×200),G: negative expression in normal gastric mucosa, H: positive expression in Well to moderately differentiated gastric adenocarcinoma, I: strongly positive expression in poorly differentiated.*

The expected sizes of PCR products were 316 bp,404 bp,329 bp for Cx43,Smad4 and PCNA respectively. The expected sizes of PCR products were 250 bp for β-actin. Complementary DNA was synthesized using AMV Reverse Transcriptase (Takara Biomedicals). PCR was performed following this procedure: briefly 300 ng of RNA was used for RT-PCR. For CDNA synthesis 50 microliters system, after adding 1.25 μL of a ribonuclease inhibitor (Takara), 10 μL of Mgcl2, 5 μL of dNTP (dATP, dCTP, dGTP, dTTP; Takara) and 2.5 μL of AMV Reverse Transcriptase (Tahara), 5 μL of 10×PCR buffer, the mixture was incubated at 30^0 C for 10 min, 42^0 C for 30 min, 99^0 C for 5 min and then at 5^0 C for 5 min as a run. The PCR mixture contained 10 μL of cDNA-10 μL of 5×PCR buffer, 24.5 μL of DEPC-water-2.5 μL of Forward and Reverse PCR primer and 0.5 μL of thermostable Taq polymerase (Takara). The amplification was done with a DNA thermal cycler (Research PCR System). After denaturation at 94^0 C for 2 min, the amplification was conducted for 35 cycles at 94^0 C for 30 s, at 59^0 C (Cx43), 58^0 C (Smad4), 56^0 C(PCNA) for 45 s, and at 72^0 C for 60 s. This was followed by a final extension for 10 min at 10^0 C. Five microliter aliquots of the product was analyzed by electrophoresis on a 2% agarose gel and visualized by UV fluorescence after being stained with ethidium bromide.

Statistical analysis

SPSS 17.0 statistical software was used for statistical analysis. Chi-square test was used to examine positive rates between groups comparison. Measurement data were expressed as mean±SD and the statistical significance was estimated by t test. Differences were considered significant when $P<0.05$.

RESULTS

Expression and localization of Cx43, Smad4 and PCNA by immunohistochemical staining

Normal gastric mucosa adjacent to gastric adenocarcinoma showed strong Cx43 and Smad4 positivity in the glandular compartment of gastric mucosa. Of the 25 cases of normal gastric mucosa, the rates of positive Cx43 and Smad4 expression were 92% and 84% respectively. While 60 cases of gastric adenocarcinoma, the rates of positive for Cx43 and Smad4 were 65% and 58%, there was statistical significance for Cx43 and Smad4 between normal gastric mucosa and gastric adenocarcinoma. The rates of positive PCNA expression were 48% (gastric adenocarcinoma) and 82% (normal gastric mucosa), there was statistical significance (Table 1). Expression of Cx43 protein showed more active patterns in well to moderate differentiation (23cases/27cases,85%), without lymph node metastasis (20/25,80%), invasion superficial muscular and submucosa (16/18,89%),TNM stages I+II (23/28,82%) gastric adenocarcinoma than Poorly differentiation (16cases/33 cases, 48%), lymph node metastasis(19/35,54%),invasion

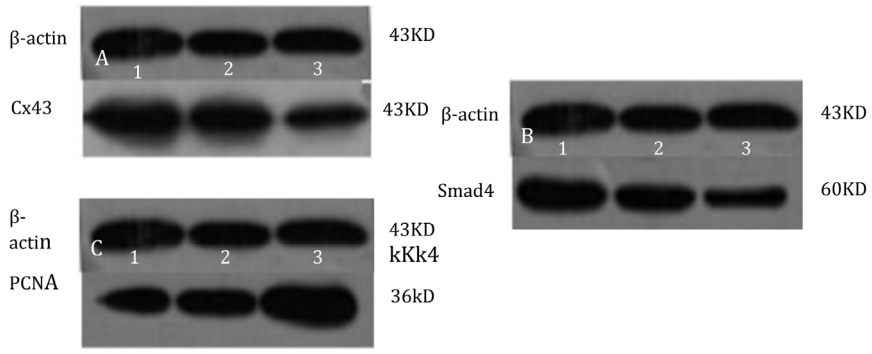

Figure 2: 1. *normal gastric mucosa, 2. Well to moderately differentiated gastric adenocarcinoma; 3. Poorly differentiated gastric adenocarcinoma. A: Western blotting for Cx43 protein, B: Western blotting for Smad4 protein, C: Western blotting for PCNA protein.*

deep muscularis and serosa(23/42,55%), TNM stages III+IV (16/32,50%). The expression of Smad4 in each group is very similar to Cx43 expression. On the contrary, higher PCNA protein expression levels in poor differentiation (30cases/33 cases,91%), lymph node metastasis(33/35,94%),invasion deep muscularis and serosa(38/42,90%), TNM stages III+IV (30/32,94%) were noted. Expression levels of Cx43, Smad4 and PCNA protein in gastric adenocarcinoma were not statistically associated with age, or gender (Table 2, fig.1).

Cx43, Smad4 and PCNA protein expression in gastric adenocarcinoma by Western blotting

To further verify the role of Cx43, Smad4 and PCNA, western blotting was employed to confirm the expression levels of these proteins. Expression is measured by the optical density (OD). Of the 25 cases of normal gastric mucosa, Cx43 protein mean OD value was 0.80±0.06. Of the 60 cases of gastric adenocarcinoma the OD value was 0.37±0.06, with statistical significance for Cx43 protein between normal gastric mucosa and gastric adenocarcinoma (Table 3). Specific expression of each group is as follows: Well-moderately differentiation (0.50±0.04), Poorly differentiation (0.23±0.03); Lymph node metastasis(0.21±0.03), without Lymph node metastasis (0.59±0.04); Invasion deep muscularis and serosa (0.29±0.02), Invasion superficial muscular and submucosa(0.55±0.03); TNM stages I+II (0.51±0.03), TNM stages III+IV (0.25±0.03), these expression differences between the groups were statistically significant(P<0.05). The expression of Cx43 in groups of age and gender is not significant. Expression of Smad4 protein in each group is very similar to Cx43 expression. Expression of PCNA is contrary to Cx43 and Smad4 expression form. Groups of Poorly differentiation, lymph node metastasis, invasion deep muscularis and serosa and TNM stages III+IV have a higher protein OD value (Table 4, fig.2).

Expression of Cx43 mRNA ,Smad4 mRNA and PCNA mRNA

The expression of Cx43 mRNA and Smad4 mRNA in carcinomatous gastric tissue were lower than that of in normal gastric tissue (P<0.05). The expression of Cx43 mRNA and Smad4 mRNA in gastric carcinoma which were well to moderately differentiated were higher than poorly differentiated gastric carcinoma (P<0.05) (Table 5). The expression of Cx43 mRNA and Smad4 mRNA in gastric carcinoma without lymph node metastasis were higher than that of lymph node metastasis (P<0.05). The expression of Cx43 mRNA and Smad4 mRNA in gastric carcinoma with TNM I+II were higher than that of TNM III+IV (P<0.05). The expression of Cx43 mRNA and Smad4 mRNA invasion deep muscularis and serosa of gastric carcinoma were higher than that of invasion superficial muscular and submucosa gastric carcinoma (P<0.05). PCNA mRNA expression for Cx43 mRNA and Smad4 mRNA has the opposite trend. Groups of Poorly differentiated carcinoma, lymph node metastasis, invasion deep muscularis and serosa and TNM stages III+IV have a higher mRNA OD value. The expression of Cx43 mRNA, Smad4 mRNA and PCNA mRNA in groups of age and gender were not statistically significant (Table 6, fig.3).

Correlation research among Cx43 and Smad4 and PCNA in the occurrence and development of gastric carcinoma

Correlation among Cx43, smad4 and PCNA in gastric carcinoma was done through analyzing experimental data. With immunohistochemistry, the correlation coefficient Cx43 of and Smad4 protein was: r=0.53(P<0.01); the correlation coefficient of Smad4 and PCNA protein was: r=-0.49 (P<0.01); the correlation coefficient Cx43 of PCNA protein was: r=-0.55 (P<0.01). By Western blotting detection the correlation coefficient Cx43 of and Smad4 protein was: r=0.88(P<0.01); the correlation coefficient of Smad4 and PCNA protein was: r=-0.79 (P<0.01); the correlation coefficient Cx43 of PCNA protein was: r=-0.75 (P<0.01). The correlation coefficient of Cx43 mRNA and Smad4 mRNA was: r=0.83(P<0.01); the correlation coefficient of Smad4 mRNA and PCNA mRNA was: r=-0.81 (P<0.01); the correlation coefficient of Cx43 mRNA

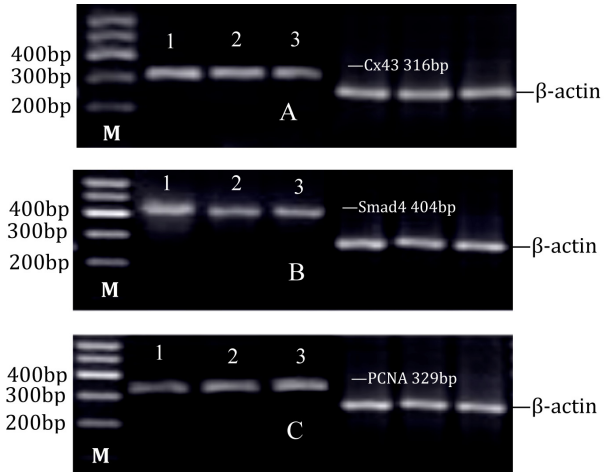

Figure 3: M. *100 bp DNA Marker, 1. normal gastric mucosa, 2. Well to moderately differentiated gastric adenocarcinoma, 3. Poorly differentiated gastric adenocarcinoma. A: RT-PCR for Cx43 mRNA, B: RT-PCR for Smad4 mRNA, C: RT-PCR for PCNA mRNA.*

and PCNA mRNA was: r=-0.76 (P<0.01).

DISCUSSION

Gap junctions which are used for the exchange of intercellular information are special membrane structures. Gap junctions are composed of the connexin subunits (connexin, Cx) across the membrane. Tumor characteristics embodied in the uncontrolled proliferation and differentiation abnormalities, there exists abnormal expression of Cx in tumors. Cx43 gap junction protein as a major participation in a variety of diseases and tumors may also have a great relationship with gastric cancer.5 Cx43 is a part of gap junction channel, in exchange of information between cells, and plays the role of cell-cell contact inhibition. Normal cells and adjacent cells were with positive expression, but contact inhibition role in tumor cells often disappeared.[6] And the most important features in cancer cells is contact inhibition disappearance, resulting in a significant decrease in Cx43, suggesting that Cx43 down regulation leading to cell gap junctional intercellular communication (gap junction intercellular communication, GJIC) abnormality or disappearance involved in gastric carcinogenesis process. GPIC as a new target in gastric cancer therapy remains to be our further research.

Our results from immunohistochemistry, Western blotting and RT-PCR technology confirm that high expression of Cx43 in normal gastric mucosa and gastric cancer tissue was significantly reduced, and the expression of Cx43 and clinical pathology of gastric cancer indicators, such as differentiation, lymph node metastasis, depth of invasion, TNM stage and so on have a great relationship with gastric cancer progression, metastasis and invasion, Cx43 expression gradually weakened, suggesting the absence of Cx43 may contribute to the progress of gastric cancer, which is consistent with Nishitani et al results.[2] In Western blotting Cx43 expression, Carystinos et al[7] study found that the expression of Cx43 in gastric mucosa tissue compared with normal gastric mucosa has been falling. Another study by Mine et al also confirmed the expression of Cx43 in volume in normal mucosa is significantly higher than gastric cancer tissue, and fully expressed in the normal gastric mucosa.[8] Only few researches have been conducted on Cx43 and its association with gastric cancer prognosis. A study done by Carystinos et al found that the connection proteins are involved in tumor metastasis, and the results also show that Cx43 may be involved in the process of tumor metastasis.[9] So whether Cx43 is involved in gastric cancer lymph node metastasis, and may be an indicator of prognosis of gastric cancer, as new targets for gastric cancer treatment, needs further research. Smad4 was the first discovered Smads family abnormal gene, most of the pancreatic cancer exists in this gene absence or mutation.[10] In the TGF-β1 signaling transduction pathway, when the receptor-binding Smads (R-Smads) and TβRI phosphorylation after separation, Smad4 then combined with the R-Smads to form heterologous oligomers, Smad4 translocation to the nucleus regulate transcription of target genes. Because it can almost combine with all activated R-Smads to form oligomers compounds, hence plays a role in the TGF-β1 signal transduction. Smads molecular abnormalities, usually characterized by pathway regulating disorder, and transcription regulation would be unbalanced.[11] Smad4 tumor inhibition, may be that it mediated the TGF-β1growth inhibition, including cut c-myc proto-oncogene expression and rise in CDK inhibitors P15 and P21 expression, thus played a entrainment in the cell cycle, that is the most significant biological effect of TGF-β1.[12] The majority of Pancreatic cancer has been found to exist in absence or mutation of Smad4 gene, and Smad4 inactivation has also been found in gastric cancer, colorectal cancer, breast cancer and lung cancer.[13] Our experimental results show that Smad4 in normal gastric mucosa tissues increased with even full expression, which means that the normal cell Smad4 in the regulation role of TGF-β1 / Smads signaling pathway is crucial. Research findings of Rodexk on the role of Smad4 keep consistent with our results.[14] In gastric clinical pathology, such as the poorly differentiated carcinomas, lymph node metastasis, invasion of deep myometrial tissue or even serosa and TNM stage into III, IV stage, Smad4 expression levels were significantly decreased. Aimin leng et al reported that Smad4 protein in gastric carcinoma was significantly lower than the adjacent tissues and Smad4 protein expression associated with the degree of differentiation of gastric carcinoma, such as in poorly differentiated gastric carcinoma tissues, Smad4 protein expression levels was significantly lower than that of well and moderately differentiated gastric carcinoma, which is consistent with our findings.

In the process of the occurrence of gastric cancer, abnormal

cell cycle regulation and uncontrolled cell proliferation are considered to be an important mechanism for gastric cancer.[15] PCNA mainly exist in the G1 phase and S phase of cell cycle, as one of the important biological indicators which reflect the cell proliferation activity, and PCNA is closely related with the biological behavior of malignant tumors, including metastasis, invasion and prognosis.[16] PCNA expression in normal gastric mucosa are weak and regular, limited to the gastric gland part zone, which may have to do with the metabolism of cells for new cells to replace aging cells. PCNA expression in poorly differentiated gastric carcinoma, lymph node metastasis, deep myometrial even serosa invasion and TNM stage into III, IV stage, were seen in higher levels, the results of which are consistent the study conducted by Maga et al, indicating that PCNA have a great relationship with the degree of invasion and prognosis.[17] This study concluded that high expression of Cx43 and low expression of PCNA was seen in normal gastric mucosa, while low expression of Cx43 and high expression of PCNA was seen in gastric carcinoma tissue. Wang Chunhong et al pointed out that the level of Cx43 expression and tumor cell proliferation ability was negatively correlated, which provide a reliable basis for our findings.[18] Cx43 further decreased expression induced gap junction intercellular communication dysfunction, malignant proliferation of gastric epithelial cells, and the Cx gene may have a negative regulation role on cell proliferation. Our experiments found Smad4 expression at high levels in normal gastric mucosa, PCNA almost no expression, Smad4 expression in gastric cancer was significantly reduced while PCNA significantly higher PCNA expression. When Smad4 reduced expression will affect the TGF-β1 signaling pathway in normal transfer, this will result in gastric epithelial cell hyperplasia, which may be a reflection of PCNA overexpression. Our experiments show that in normal gastric mucosa Cx43 and Smad4 keep a state of high expression and are down regulated in gastric carcinoma. Research done by Ping Dai et al show that TGF-β and Smad signaling pathway was through the medium of Cx43, found the TGF-β function relation with the Cx43 activities by Smad2/3, microtubule and Cx43 interactions.

CONCLUSION

Mucin stains like Alcian blue and PAS helps to identify malignant cases and specificity will be increased to 96.8% combining the Alcian blue positivity in cases of PSA >10ng/ml. PSA density may be helpful in differentiating nodular hyperplasia of prostate, prostatitis with carcinoma prostate. A larger population based prospective study is required to find out the cutoff value for taking biopsy after a screening PSA for our endogenous population so that cases of prostatic carcinoma are identified in earlier stage and treatment can be started promptly.

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