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Correlations of β-catenin, Ki67 and Her-2/neu with gastric cancer

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ABSTRACT

Objective: To study the clinical pathologic characteristics of β-catenin, Ki67 and Her-2/neu in gastric cancer and the correlation of β-catenin and Ki67 to the protein expression and gene conditions of *Her-2/Neu*. **Methods:** The protein expression of β-catenin, Ki67 and Her-2/Neu was detected by immunohistochemistry in 101 cases of gastric cancer and the gene conditions of *Her-2/Neu* by fluorescence in situ hybridization (FISH). **Results:** The protein expression of β-catenin, Ki67 and Her-2/Neu had close relationship with the clinical pathologic characteristics of gastric cancer. The β-catenin had obvious correlation to the differentiation, infiltration and lymphatic metastasis of the gastric cancer ($P<0.05$). The Ki67 had close relationship with the tumor-node-metastasis staging staging of gastric cancer ($P<0.05$). Her-2/Neu had close relationship with the differentiation and tumor-node-metastasis staging of gastric cancer ($P<0.05$) but had no relationship with the infiltration and lymphatic metastasis of the gastric cancer ($P>0.05$). The protein expression of Ki67 had significantly positive correlation with the protein expression and gene amplification conditions of Her-2/Neu ($r=0.567$, $P<0.05$ for protein, $P<0.05$ for gene). **Conclusions:** Combined detection of β-catenin, Ki67 and Her-2/neu can be used as a reliable method to help the observation of biological behavior, diagnosis and prognosis of gastric cancer, and Ki67 can be used to serve the preliminary screening of *Her-2/Neu* gene state.

1. Introduction

Gastric cancer, known as a malignant tumor[1], is characterized by cell invasion and metastasis and is also a main cause of death[2]. Ki67 is widely used as a marker of cell proliferation and its function is closely associated with chromatin. β-catenin exhibits the roles of signal transduction and cellular adhesion in Wnt signaling pathway. Amplification of *Her-2/neu* gene is the key to the efficacy of molecular targeted drugs. We investigated the correlations of β-catenin, Ki67 and Her-2/neu with the clinical characteristics of gastric cancer and analyzed the relationship between the expression of β-catenin, Ki67 and Her-2/neu proteins. Reliable reference indices were also searched to screen *Her-2/neu* gene state.

2. Materials and methods

2.1. General data

Cancerous gastric tissue samples were surgically resected from 101 gastric cancer patients who received treatment at Shandong Provincial Hospital, China between March 2012 and May 2013. Simultaneously, fresh stomach mucous membrane tissues, which was confirmed pathologically free of carcinomatous change, were harvested from the

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cutting edge of diseased foci of 76 gastric cancer patients and used as control. The included patients did not receive any anti-tumor treatment before surgery and provided complete clinical record document. Following hematoxylin-eosin staining, all tissue sections were diagnosed as adenocarcinoma by two experienced physicians in consensus. The gastric cancer patients, 52 males and 49 females, were at the age of 57 (range from 34 to 80) years. The size of tumor was ≥5 cm in 70 patients and <5 cm in 31 patients. According to the WHO criteria, tumor cells were well- and medium-differentiated in 51 patients and poorly-differentiated in 50 patients. As per the pathological tumor-node-metastasis staging (pTNM) system issued by the American Joint Commission for Cancer Staging (2011), tumors were staged as I/II in 58 patients and as III-IV in 43 patients. According to the depth of invasion, tumors invaded the serosa in 57 patients and had invaded but not penetrated the serosa in 44 patients. Lymph node metastasis was present in 68 patients but not in 33 patients.

2.2. Main instruments and reagents

The experimental instruments used in this study were provided by Department of Pathology, Shandong Provincial Hospital, China. Other instruments included pa embedding apparatus (Germany), microtomes (LI A BM2135; AO, USA), optical microscope (BX-50; Olymp Japan), constant temperature baker (S Yuej Medical Instrument Factory, China), a n situ bridize (ThermoBriteTM, StatSpin, USA). Im h reagents were purchased from jing Zh han Golden Bridge Biotechnology Co., China. *Her eu* gene fluorescence in situ hybrid ation (H) detection kits and reagents were purch d from Beiji inpujia Medical Science and Techn gy Co. td., China.

2.3. Immunohistochem stain assay

Im ohist hemical aining was performed using strepta kit according to the manufacturer's instructio BS, rather than primary antibody, was taken as a negativ ntrol. Through an optical microscopy, β-catenin- and Ki67-positive cells were counted in five fields of view randomly selected from each section. The percentage of β-catenin- or Ki67-positive cells ≤10% was considered as 0 point, 11%-50% as 1 point, 51%-75% as 2 points, and >75% as 3 points. No staining was scored 0, light brown 1 point, brown 2 points, and dark brown 3 points. The final score was obtained by sum of the above two scores: 1-2 points were considered as "1+", 3-4 points as "2+" and "5-6" points as "3+". Finally, sample with a total score of 0 was considered as negative, while sample with a total score of 1+ to 3+ as positive. Her-2/neu immunoreactivity was scored according to HercepTest guidelines. Her-2/neu-positive cells exhibited brown yellow staining.

2.4. FISH detection

FISH detection was performed in strict accordance with kit instruction. Thirty cells were counted for calculation of ratio value according to the formula:

Ratio = Number of red signals in 30 nuclei/Number of green signals in 30 nuclei.

Ratio <1.8 was considered neg e, indic g absence of *Her-2/neu* gene amplificatio the test sample; Ratio >2.2 was considere positive dicati presence of *Her-2/neu* gene am ificati in t ted sample; 1.8≤Ratio≤2.2, count ells repeat FISH test.

2.5. Statistical nalysis

Statistical analysis performed using SPSS 17.0 software. Di ence comparison w carried out using Chi-square test a Fisher's ex t test. Correlation analysis was conducted u Spearma rank correlation. A level of $P<0.05$ was con ed st tically significant.

3. Results

3.1. Expression of β-catenin, Ki67 and Her-2/neu proteins in cancerous gastric tissue

As shown by the immunohistochemical staining, the positive expression of β-catenin and Ki67 was located in the nuclei (Figure 1A and 1B), and that of Her/neu was found in the cellular membrane (Figure 1C). The positive cells were stained light or dark brown. The expression of *Her-2/neu* gene was also detected by FISH. The red signals of *Her-2/neu* gene amplification (positive) were distributed in clusters or diffusion spots (ratio >2.2; Figure 2A), while the signals were distributed in diffusion spots (ratio <1.8; Figure 2B) without *Her-2/neu* gene amplification.

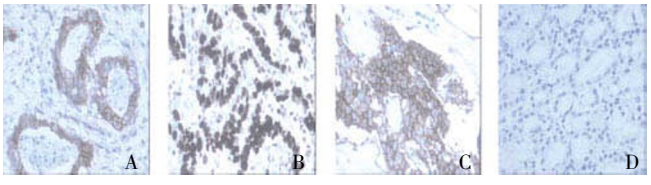

Figure 1. Expression of β-catenin, ki67 and Her-2/neu in the tested samples detected by immunohistochemistry (×200).
(A) β-catenin-positive expression (3+); (B) Ki67-positive expression (3+); (C) Her-2/neu-positive expression (3+); (D) Her-2/neu-negative expression.

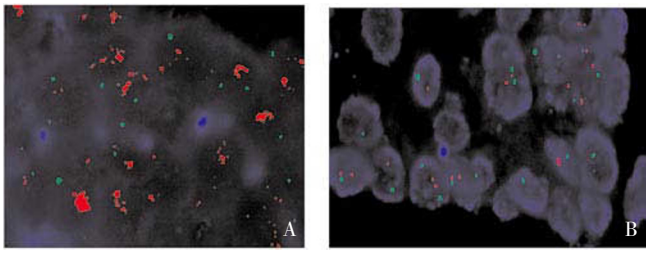

Figure 2. Amplification of *Her-2/neu* gene in the tested sample detected by in situ hybridization (×200).
(A) With *Her-2/neu* gene amplification (ratio >2.2); (B) Without *Her-2/neu* gene amplification (ratio <1.8). *Her-2/neu gene* is shown by red signals and centromere 17 by green signals

Among the 101 patients with gastric cancer, the number of β-catenin-positive patients scored 0, 1+, 2+ and 3+ was 26(25.7%), 4(4%), 29(28.7%) and 42(41.6%), respectively, while the number of Ki67-positive patients was 20(19.8%), 24(23.8%), 15(14.8%) and 42(41.6%), respectively. The number of Her-2/neu-positive patients scored 2+ and 3+ was 6(6.0%) and 10(10.0%), respectively. The percentage of β-catenin-, Ki67-, and Her-2/neu-positive patients among the 101 gastric cancer patients was 74.3%(75/101), 80.2%(81/101) and 15.8%(16/101), respectively, which was significantly higher than that of the control group [36.8%(28/76), 25.0%(19/7[illegible] 1.3%(1/76), $P<0.05$].

3.2. Correlations of β-catenin, Ki67 and Her-2/neu expression with the clinical pathological characteristics of gastric cancer

The expression of β-catenin, Ki67 and Her-2/neu was not correlated with the gender, age, and tumor size of patients with gastric cancer. The expression of β-catenin and Ki67 were significantly correlated with degree of tumor cell differentiation, depth of tumor cell invasion and lymph node metastasis ($P<0.05$). The β-cate[illegible] expression was not correlated with pTNM stage. Th[illegible]er-2[illegible] expression was significantly correlated with [illegible]M stage (P [illegible]05), but it was not correlated with the [illegible]th of [illegible]r cell i[illegible]asion and lymph node metastasis (T[illegible]e 1).

3.3. Correlations [illegible]Ki67 [illegible]in exp[illegible]on with Her-2/neu protein expres[illegible]and gene s[illegible]

The Ki67 protein [illegible]ression was positively correlated wi[illegible]2/neu protei[illegible]xpression ($r=0.567$, $P<0.05$) and p[illegible]tively corr[illegible]ated with *Her-2/neu* gene state ($r=0.304$, P [illegible]05). The [illegible]munohistochemistry results showed that [illegible]e rate of Her-2/neu expression (21.4%) was significantly higher in the 9 gastric cancer patients [illegible]ng with the positive expression of Ki67 (3+) and Her-2/neu (3+) than in other types (Table 2).

Table 1
Correlations of β-catenin, Ki67 and [illegible]-2/neu wi[illegible] clinical pathological characteristics of gastric cancer.

Characteristic		β-catenin			Ki67			Her-2/neu		
		Positive rate (%)	χ^2	P	Positive rate (%)	χ^2	P	Positive rate (%)	χ^2	P
Sex	Male	[illegible]1.15(37/52)	0.540	0.502	84.62(44/52)	0.920	0.445	17.31(9/52)	0.173	0.444
	[illegible]ale	77.55(38/49)			75.51(37/49)			14.29(7/49)		
Age (years)	⩾60	72.50(29/40)	0.107	0.744	77.50 (31/40)	0.304	0.616	12.50(5/40)	0.555	0.581
		75.41(46/61)			81.97(50/61)			18.03(11/61)		
Tumo[illegible]meter	⩾5	72.86(51/70)	0.234	0.806	81.43(57/70)	0.217	0.787	17.14(12/70)	0.290	0.770
	<5	77.42(24/31)			77.42(24/31)			12.90(4/31)		
Degree of tumor ce[illegible]rentiation	Well/medium	60.78(31/51)	9.783	0.003	70.59(36/51)	5.991	0.023	7.84(4/51)	4.944	0.031
	Poor	88.00(44/50)			90.00(45/50)			24.00(12/50)		
Depth of tumor cell invasion	Not invade serosa	64.91(37/57)	5.978	0.021	71.93(41/57)	5.632	0.018	14.04(8/57)	0.320	0.594
	Invade serosa	86.36(38/44)			90.91(40/44)			18.18(8/44)		
Lymph node metastasis	Yes	80.88(55/68)	4.778	0.330	86.76(59/68)	5.561	0.031	14.71(10/68)	0.201	0.772
	No	60.61(20/33)			66.67 (22/33)			18.18(6/33)		
Tumor-node-metastasis staging	I+II stages	79.31(46/58)	1.820	0.250	72.41(42/58)	5.198	0.023	8.62(5/58)	5.328	0.028
	III+IV stages	67.44(29/43)			90.70(39/43)			25.58(11/43)		

Table 2
Correlations of Ki67 protein expression with Her-2/neu protein expression and gene stage.

Ki67 protein expression	Her-2/neu protein expression		*r*	*P*	Amplification of *Her-2/neu* gene		*r*	*P*
	++	+++			Yes	No		
++	2	1	0.567	0.043	3	12	0.304	0.022
+++	1	9			2	40		

3.4. Analysis on Her-2/neu protein expression and Her-2/neu gene state

Through FISH, the amplification of *Her-2/neu* gene was detected in 14 patients, with a gene amplification rate of 13.9%(14/101). According to the Her-2/neu protein expression, the patients were divided into two groups: Her-2/neu (2+/3+) and Her-2/neu (-/+). The amplification rates of *Her-2/neu* gene in the sanples with Her-2/neu (3+) and Her-2/neu (2+) were 90.0%(9/10) and 83.3%(5/6), respectively. In the tested samples with Her-2/neu (-/1+), no amplification of the *Her-2/neu* gene was detected (0/33 and 0/52, respectively). The detection results of patients with Her-2/neu (2+/3+) by immunohistochemistry was 87.5% consistent with the amplification resluts of *Her-2/neu* gene by FISH.

4. Discussion

Abnormal or up-regulated expression of [illegible] in lea[illegible] to the occurrence of diseases[3]. It has [illegible]n evid[illegible]ced tha[illegible] β-catenin influences the biological beha[illegible]rs [illegible] cells[4] and its down-regulation i[illegible]roves ce[illegible]trastructure, promotes apoptosis and in[illegible]ell prolife[illegible]n[5]. We found β-catenin was si[illegible]nificantly [illegible]related with degree of tumor cell differe[illegible]tion, depth of [illegible]or cell invasion and lymph node [illegible]etast[illegible] (P<0.05), suggesting that abnormal β-caten[illegible]ssion in [illegible]he gastric cancer tissue helps gast[illegible]ncer [illegible] to [illegible]ain stronger invasive and metast[illegible] abili[illegible] and th[illegible]dicates poor prognosis.

At p[illegible]ent, [illegible] been considered as a reliable index for evalu[illegible] cell proliferation and malignant potentials and predicting c[illegible]r recurrence[6-7], and its expression level is of important clinical significance for tumor treatment and metastasis[8]. It also shows a predictive value in adjuvant chemotherapy. In our study, the Ki67 expression rate was 70.6% in the well- and medium-differentiated gastric cancer, 90% in the poorly differentiated gastric cancer, 68.6% in the serous layer-infiltrated tissues and 90.9% in the non-infiltrated tissues. The results suggest that changes in proliferative activity of tumor cells are related to the progression of gastric cancer.

Her-2/neu, also called C-erbB-2, shows similar functions as Ki67 and both can promote mitosis. In this study, the positive expression rate of Her-2/neu was 15.8%, which is in accordance with a previous report[9]. The positive expression rate of Her-2/neu was 3.4[illegible] in the well- and medium-differentiated tissu[illegible]d poorly [illegible]erentiated tissue respectively, suggesting tha[illegible]h expres[illegible]n level of Her-2/neu is a marker of [illegible]alignant [illegible]or. [illegible]eover, the amplification rate of [illegible]-2/ne[illegible]gene i[illegible]used with the increasing grades ind[illegible]e stat[illegible]of *Her-2/neu* gene becomes more i[illegible]able wit[illegible]reas[illegible]malignance of gastric cancer.

The appli[illegible]er-ceptin, the monoclonal antibody aga[illegible]er-2/neu, [illegible]e treatment of gastric cancer a[illegible]cts more attention and disputes. Trastuzumab (trade n[illegible]e: herce[illegible]n), a molecular targeted drug, is only effi[illegible]ve in t[illegible]or patients presenting with *Her-2/neu* gene a[illegible]ation. Her-2/neu (2+) was previously used as [illegible]eference for FISH detection of *Her-2/Neu* gene, but [illegible]egative FISH detection results also exist in subjects with Her-2/neu (3+). To predict the *Her-2/neu* gene state of tumor patients more simply and accurately, we statistically analyzed the present results and found that the expression of Ki67 protein was positively correlated with the protein expression and gene amplification of Her-2/neu (r=0.567, P<0.05 for protein; r=0.304, P<0.05 for gene). The *Her-2/neu* gene amplification analyzed by FISH was consistent with the protein expression detected by immunohistochemistry, with a K value of 0.879, demonstrating that the protein expression and gene state had good consistency. This is in line with previous reports. However, contrary results have also been reported, which may be caused by differences in experimental methods, interpretation criteria and sample size. Further investigation is needed to eliminate this conflict.

Our results demonstrated that the protein expression and gene amplification of Her-2/neu were closely related to the expression of Ki67 protein. Previous researches show that Her-2/neu protein does not act independently but exhibits synergic and inhibitory effects together with other factors. Further studies are required to investigate whether the proliferative activity of gastric cancer cells promotes the *Her-2/neu* gene amplification and whether there is a

regulatory relationship between Ki67 and Her-2/neu. We can speculated that the Ki67 protein expression, in addition to Her-2/neu (2+) protein expression, may be selected as another assistant reference in screening *Her-2/neu* gene state by FISH.

Taken together, the expression of β-catenin, Ki67 and Her-2/neu is closely related to the clinical pathological characteristics of gastric cancer. Combined detection methods can provide an overall reference evidence for understanding the nature, clinical diagnosis and prognosis of tumors. Her-2/neu combined with Ki67 can be used as a predicator for screening *Her-2/neu* gene state by FISH, which will increase detection efficiency of *Her-2/neu* gene amplification.

Conflict of interest statement

We declare that we have no conflict of interest.

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