HER2 protein expression correlates with Lauren classification and P53 in gastric cancer patients

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Research

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Abstract

Background and objective: Human epidermal growth factor receptor 2 (HER2) is a key pathological characteristic in gastric cancer patients. However, the clinical significance of HER2 protein expression in gastric carcinoma remains controversial. The purpose of the study is to analyze the clinicopathological characteristics of HER2 protein expression, Lauren classification and P53 expression and evaluate the clinical significance of the HER2 protein expression.

Methods: A total of 176 consecutive patients were recruited prospectively between January 2014 and December 2016 in The Second Affiliated hospital of Zhejiang University School of Medicine. Histological analysis was performed on resected tissue for HER2 protein expression by immunohistochemistry (IHC). The patients with IHC grade 2+ were analyzed by fluorescence in situ hybridization (FISH) to assess the expression status of HER2 protein. Moreover, standardized criteria of HER2 protein expression in gastric cancer was used in this study. Additionally, the expression status of HER2 protein and clinicopathological features were analyzed by Chi-square (c^2) test. All statistical analyses were conducted using the SPSS 22.0 statistical software program (IBM Corp., SPSS statistics, Chicago, IL).

Results: A total of 176 gastric cancer patients were enrolled in this study. Intratumorally heterogeneity of HER2 protein overexpression was 42 of 176 cases with IHC grade 2+ accompanied with FISH positivity and IHC grade 3+. HER2 protein expression correlated with tumor differentiation ($p < 0.001$), Lauren classification ($p = 0.001$), Borrmann type ($p = 0.003$) and P53 expression ($p < 0.001$). Overall survival (OS) was not analyzed because the follow-up duration was too short and the high rate of missed interview.

Conclusions: The overexpression of HER2 protein was determined in 23.9% of the cases and significantly related to Lauren intestinal subtype and P53 expression.

Introduction

Gastric cancer (GC) is the third leading cause of cancer-related death worldwide after lung and liver cancer[1]. Although the incidence of GC has decreased worldwide, more than half of the cases occur in Eastern Asia, leading to a significant public health problem in those countries, including China[1,2]. Despite advanced methods of early detection and the multidisciplinary treatment of advanced GC, the 5-year survival still remains unsatisfactory, although better results are achieved in Japan and South Korea[3].

HER2 protein is a transmembrane receptor tyrosine kinase that plays an important role in the invasion and progression of GCs[4,5]. Overexpression of the HER2 protein was reported in 7-34% of GC patients and was considered an inversely prognostic factor[6-8]. It is important to note that the median overall survival time was longer in HER2 protein positive patients who received trastuzumab plus chemotherapy than in those who received chemotherapy only in phase 3 clinical trial (Trastuzumab for Gastric Cancer or ToGA)[9]. However, some studies found no relationship between HER2 protein overexpression and survival[10-12]. Also, in such studies the HER2 protein expression status was measured by

immunohistochemistry (IHC) and the (normal, deletion or amplification) status was evaluated by fluorescence in situ hybridization (FISH) analysis; those patients with IHC 3+ or IHC grade 2+ and FISH+ were considered HER2 protein overexpression. Recently, several studies reported the clinical characteristics and prognosis relating with HER2 protein overexpression in GCs[2,13,14]. However, the conclusions drawn from such studies were varied. Therefore, the present study aimed at assessing HER2 protein expression in a cohort of Chinese patients with GC, evaluating the HER2 protein heterogeneity of GC and determining the correlation of the HER2 protein expression with the clinicopathological features in patients with GC.

Materials And Methods

Patients: This retrospective study involved in a cohort of 176 GC patients who underwent curative surgery for primary GC at The Second Affiliated Hospital, College of Medicine, Zhejiang University, between January 2014 and December 2016. Patients enrolled in the study met the following criteria: (1) patients were pathologically diagnosed as GC after gastrectomy by two experienced physicians; (2) received no neoadjuvant chemotherapy or pre-radiotherapy; (3) adequate paraffin-embedded tumor tissue sample for HER2 protein detected and clinicopathological analysis; (4) no concurrent malignancy; (4) complete set of medical records for analysis were available.

The following demographic and clinicopathological features were recorded and categorized: age (<60 and ≥60 year-old), sex, tumor markers, histological grade, Lauren's classification (intestinal, diffuse, mixed), location of tumor (antrum, body, proximal), tumor size (≤5 and >5 cm), lymphovascular and muscle invasion, liver metastasis, Borrmann type, pathologic TNM stage (according to 8th American Joint Committee on Cancer (AJCC) Tumor-Node-Metastasis (TNM) stage). It was clearly established that 56 patients had stage I disease, 44 had stage II disease, 61 had stage III disease, 15 had stage IV disease. The clinicopathological data were obtained from the hospital information system (Electronic Medical Record System, EMRS).

Immunohistochemistry: For all patients, the expression of Her2 protein, Ki67 and P53 were measured by two experienced pathologists by IHC and without advanced knowledge of clinical features. Histopathological parameters were measured through representative 4-μm thick paraffin-embedded tissue sections. The IHC staining was performed using a primary polyclonal rabbit antibody (DAKO, Glostrup, Denmark) against Her2 protein on a Ventana Benchmark XT automatic staining system (Ventana Medical Systems, Tucson, AZ, USA), according to the manufacturer's instructions. To determine the expression of Her2 protein, the IHC scoring criteria proposed by Hoffmann et al. was used as follows[7]. The sorting grades are: grade 0: no membrane staining or membrane staining in less than 10% of the tumor cells; grade 1: faint or barely perceptible membranous reactivity in 10% or more of tumor cells; grade 2: weak to moderate reactivity in at least 10% of the cells, with staining across the lateral and basolateral membrane; grade 3: strong staining of the complete membrane in more than 10% of the tumor cells[9]. The score of IHC 0 and 1+ were regarded as negative for Her2 protein expression, and IHC 3+ was considered positive. As for IHC 2+ patients, it is necessary to evaluate the Her2 protein

normal/deletion/amplification status by FISH analysis[15]. FISH analysis was performed using the PathVysion®Her2 DNA Probe kit (LSI®Her2/neu Spectrum Orange™/chromosome 7 centromere probe (CEP)®17 Spectrum Green; Abbott Vysis, Downers Grove, IL, USA) and the process was performed according to the kit manufacturer's instructions. Her2 protein was considered as overexpressed when the FISH showed the Her2:CEP17≥2 and the remaining were defined as negative. The Ki67 and P53 were evaluated semi-quantitatively. On the basis of the level of nuclear staining, the scoring was initially classified using a scale from 0-4, as follows: 0, less than 10% staining; 1, 11-25% staining; 2, 26-50% staining; 3, 51-75% staining; 4, 76-100% staining. Finally, the cut-off value was established as previously described by Al-Moundhri et al.[16]. When the staining of Ki67 was more than 25% that the sample was considered as positive for analysis. As for P53, the cut-off value was established at 10% staining. The similarity is that when the staining of P53 >10% and the staining of Ki67 >25% the sample displayed obvious nuclear staining.

Statistical analysis: Pearson's chi-square (c^2) test or Fisher's exact test was used to compare the differences among groups. P value <0.05 was considered statistically significant. All statistical analyses were conducted using the SPSS 22.0 statistical software program (IBM Corp., SPSS statistics, Chicago, IL).

Results

Among the 176 patients enrolled in this study, 129 were male and 47 were female, with a median age of presentation of 63.3 years (range, 34-87 years). All patients underwent radical surgery for carcinoma of the stomach. Most tumors, 72.7% (n=128), were located in the distal stomach, while 27.3% (n=48) were located in the proximal stomach. The majority, 59.1% (n=104), were histologically of the intestinal subtype and Borrmann type III (67.0%, n=118) at presentation. Only 25.6% (n=45) were early stage GC based on inspection of the surgical specimen tissues.

The expression of HER2 protein was measured by the methods mentioned above and the results are showed in Table 1. A total of 23.9% (n=42) of the patients were considered positive for HER2 protein expression, while 76.1% (n=134) of the patients were considered negative (Figure 1). The results of the univariate analysis, comparing the clinicopathological features of GC patients with the HER2 protein status are presented in Table 2. HER2 protein-positive GCs is prominent among patients older than 60 years of age, 27.2% (31/114), also predominated in males 25.6% (33/129). Also, HER2 protein positivity was more commonly detected in distal tumors 24.2% (31/128), though this was not statistically significant (p=0.857).

Table 1.
IHC combined with FISH defining HER2 protein expression status.

	HER2 negative			HER2 positive	
	0	1+	2+/Fish⁻	2+/Fish⁺	3+
No. (%)	74(42.0)	38(21.6)	22(12.5)	6(3.4)	36(20.5)
Sum. (%)		134(76.1)		42(23.9)	

Table 2.

Correlations of HER2 protein expression status and Lauren classification with clinical pathological characteristics of GC.

	HER2 status		P	Lauren classification			p
	Positive(n=42)	Negative(n=134)		Intestinal(n=104)	Diffuse(n=38)	Mixed(n=34)	
Sex			0.376				0.491
Male	33	96		78	25	26	
Female	9	38		26	13	8	
Age			0.160				0.105
≥60	31	83		68	20	26	
≤60	11	51		36	18	8	
Primary tumor			0.655				**0.001**
T1	14	34		39	4	5	
T2	6	19		15	3	7	
T3	18	60		42	19	17	
T4	4	21		8	12	5	
Lymph nodes metastasis			0.446				0.189
N0	22	52		52	12	10	
N1	6	20		14	6	6	
N2	5	21		13	5	8	
N3	9	41		25	15	10	
Distant metastasis			0.950				0.136
M0	39	121		95	32	33	
M1	3	12		8	6	1	
Stage			0.198				**0.008**
1	19	37		44	5	7	
2	9	35		22	10	12	
3	11	50		30	17	14	
4	3	12		8	6	1	
Tumor differentiation			**<0.001**				**<0.001**
Well	17	19		30	3	3	
Moderate	19	56		58	2	15	
Poor	6	59		16	33	16	
Lauren classification			**0.001**				
Intestinal	35	69					
Diffuse	3	35					
Mixed	4	30					
Tumor location			0.857				0.824
Proximal	11	37		30	9	9	
Distal	31	97		74	29	25	
Borrmann			**0.003**				**<0.001**
1	7	6		11	0	2	
2	14	25		29	3	7	

Most patients, 72.7% (n=128), presented with advanced (T2 and above) tumors and 21.9% (n=28) patients were HER2 protein positive. Additionally, among the 45 patients with early stage GC, 31.1% (n=14) of the patients were HER2 protein positive, but it was not statistically significant (p=0.186). Also, among the 74 patients without lymph node metastasis 29.7% (n=22) were HER2 protein positive. Meanwhile, among the 102 patients whose resected gastrectomy specimens showed lymph node metastasis, 19.6% (n=20) were HER2 protein positive. As for M (metastasis) stage patients, cross-sectional image-based staging revealed that 8.52% (n=15) patients were in M1 stage (distant metastases present) while 20.00% (n=3) of these patients were HER2 protein positive. In our research, 72.2% (n=127) of the patients were P53 positive and the patients with positive P53 expression have a higher proportion of negative HER2 protein expression ($p<0.001$). The expression of P53 immunohistochemistry was shown in the figure below (Figure 2).

According to the univariate analysis, there was a significant association (p<0.001) between HER2 protein positivity and high histological differentiation of the tumor. Specifically, 20.5% (n=36) of the patients had histologically highly differentiated tumors, while 47.2% (n=17) of the patients expressed HER2 protein, which means that the expression of HER2 protein is more common in GC patients with high-grade differentiated tumors. Similarly, most tumors, 59.1% (n=104), belonged to the intestinal subtype and the intestinal subtype expressed higher level of HER2 protein (33.7%, n=35). A significant correlation was observed between HER2 protein expression and the histological subtype of the GC (p=0.001). As shown in Table 2, HER2 protein positivity was involved with the Borrmann classification (p=0.003), vascular invasion (p=0.036), and P53 expression (p<0.001). However, there was not statistically significant association between HER2 protein positivity and Ki67 expression (p=0.095), nerve invasion (p=0.075), tumor size (p=0.952), tumor early stage (p=0.186). Also, a relationship was found between the Lauren classification and clinicopathological characteristics of the GC patients, as shown in Table 2. Specifically, the Lauren classification was associated with primary tumor (p=0.001), TNM stage (p=0.008), tumor differentiation (p<0.001), Borrmann type (p<0.001), vascular invasion (p=0.004), nerve invasion (p=0.001), and GC early stage (p=0.001).

Discussion

Despite a worldwide decline in its incidence, GC remains one of the most common malignancies in China, where GC incidence (29.9 new diagnoses per 100,000 people) remains high[17]. Currently, according to epidemiological research worldwide GC predominantly affects elderly males[17,18]. In the present study, 64.8% (114/176) of the patients were 60 years old or older. The Lauren classification, tumor location, HER2 protein expression status, tumor differentiation and size are essential clinicopathological features which have been reported in the research of GC. In our study, HER2 protein and P53 expression status were two important clinicopathological characteristics that were investigated to show the association between the expression of these genes with other GC-related factors.

In the current study, we found an overall HER2 protein positive rate of 23.9% for the GC patients. Such finding is similar to those of various other studies from American and European researchers who have reported gastric cancer HER2 protein positive rates ranging from 10 to 22.8%[4,5]. However, some Asian studies have reported rates ranging from 11.7 to 15.74%[14,19,20]. The importance of the HER2 protein status in GC remains controversial and more and more researchers are studying the significance of HER2 protein expression in GC. In general, Lauren classification can be divided into the intestinal subtype, diffuse subtype and mixed subtype. In this study, there was a significant relationship between HER2 protein expression and the intestinal subtype of GC. Specifically, the intestinal subtype patients were more likely to be HER2 protein positive than the other two subtypes. Our results are also similar to those of most of the reported studies, which revealed that the intestinal histological subtype was predominant in HER2 protein positive patients[12,13,15,21]. In addition, HER2 protein expression was found to be associated with differentiated histology. High rates of HER2 protein positive GCs were detected among well-differentiated and moderately-differentiated GCs when compared with the poorly differentiated GCs.

This result is similar to that reported by Oh et al. indicating that HER2 protein overexpression was associated with intestinal subtype and well-differentiated and moderately-differentiated GC tumors[22]. In addition, the positive rate of HER2 protein is 31.3% higher in gastric cancer patients with negative vascular invasion (25/80) and the result has obvious statistical significance (p=0.036). Some studies have reported findings similar to those of this study regarding the anatomical location of the tumor, indicating that there is no difference between distal and proximal GC tumors[2,11,23]. The efficacy of trastuzumab in breast cancer therapy has led to emerging interest in its therapeutic effect in patients with HER2 protein positive GC. The ToGA trial, a randomized, controlled, multicenter, phase III study, was designed to evaluate the efficacy of trastuzumab (an anti-HER2 protein drug) in association with chemotherapy for the therapy of advanced gastric carcinoma[9]. The expression rate of HER2 protein in the ToGA study was 22.1%, which is similar to our findings. Additionally, also similar to our study, the ERBB2 expression was an advantage in the intestinal GC type compared with the other two types. However, the ToGA study revealed that HER2 protein expression was correlated with the location of the tumor, indicating that the tumor was prevalent in the proximal of stomach. The finding of no significant correlation between HER2 protein expression and the location of the tumors in our study could be explained by the small number of enrolled patients in our research.

In China, a study conducted by Qiu et al. in 2014, analyzed the immunohistochemical expression in 838 GC cases and found prevalent HER2 protein expression in 11.2%[15]. They found predominant HER2 protein expression in intestinal GC type, and they detected a significant association between the expression of HER2 protein and the proximal GC. The frequency of HER2 protein expression was much lower than that in our study. The apparent discrepancies between the various other studies and our study can be reconciled if we consider the immunohistochemical method used and the differences among the enrolled patients. A second study conducted at Sun Yat-sen University by Liu et al. in 2016 found HER2 protein expression in 40.3% of 678 patients analyzed[24]. Such a high rate of HER2 protein expression may be due to the enrolled patients, which only included stage I-III GC patients, while our study included patients with I-IV grade of GC according to the eighth edition of the American Joint Committee on Cancer TNM staging system. In addition, both studies concluded that HER2 protein expression was significantly correlated with tumor histological classification and HER2 protein was preferentially expressed in the intestinal subtype of GC tumor. Moreover, the GC intestinal subtype was seen more in well differentiated carcinoma and lower stage of tumors. Accordingly, it is not difficult to explain why the study by Liu et al. found higher HER2 protein expression compared to our current study. A review of the previous research reveals that the Lauren classification was considered as one of the most important predictive factors of prognosis in GC[25]. Also, the intestinal subtype and well differentiated tumor cells had a consequential relationship with a better prognosis[25,26].

HER2 protein expression was regarded as the most crucial advancement in gastric carcinoma over the coming years at the American Society of Clinical Oncology (ASCO) annual meeting in 2014. In the current study, we compared our results to GC clinicopathological characteristics. The expression rate of P53 by IHC analysis was 72.2% (127/176). Additionally, the HER2 protein expression status was significantly

correlated with the P53 expression (p<0.001). Also, according to previous studies, there is a correlation between HER2 protein expression status and P53 nuclear staining, but further research on the relationship is warranted[27]. Moreover, P53 expression also significantly correlated with some clinicopathological features: HER2 protein expression (p=0.04), younger patients below 60 years old (p=0.03), tumors lager than 5 cm in size (p=0.01), and Ki67 (p=0.0001)[16]. Furthermore, in the current study, the overexpression of P53 was considered to be an independent prognostic factor[28]. Younger patients with larger tumor and high proliferation index, as measured by the level of Ki67 expression, indicate a more aggressive biological behavior of tumor cells and have worse prognosis[29,30]. However, Moundhri et al. reported that there was no relationship between P53 with sex, tumor location, Lauren classification, T-stage and lymph node metastasis[16]. Similarly, we found that there was an obvious significant difference between the HER2 protein expression and P53 expression (p<0.001). However, in our current study we did not find significant correlation between P53 and age, sex, tumor size, Ki67 and some additional clinicopathological factors.

Our study has several inherent limitations. (1). It is a retrospective study rather than a prospectively designed study and the data is limited. (2). Our research was a single-center investigation and the selection bias was inevitable, while the benefit of single-center is that we could ensure the accuracy and consistency of the research methods and data. (3). Since the follow-up time of the enrolled patients was too short and the high rate of missed interview, we could not perform a correlation analysis of the prognosis and obtain an accurate correlation between prognosis and clinicopathological factors. (4). The potential mechanism of HER2 protein overexpression as well as that of P53 overexpression in intestinal type GC require further experimental study.

Conclusions

Our IHC-based study found an HER2 protein expression rate of 23.9% in the GC patients. HER2 protein overexpression is more likely to occur in Lauren's intestinal subtype and P53-negative GC patients. The HER2 protein expression status may be related with the prognosis of GC patients, but further prospective studies are required to confirm this possibility.

Abbreviations

HER2: Human epidermal growth factor receptor 2; P53: Tumor protein p53; IHC: Immunohistochemistry; FISH: Fluorescence in situ hybridization; OS: Overall survival; GC: Gastric cancer; Ki67: Marker of proliferation Ki-67.

Declarations

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Author's contributions

The first author of this manuscript is Yiming Chu. Design, drafting and revising of the work, acquisition and analysis of data were done by Yiming Chu and Hongbo Li. Conception, design, and final approval of the version to be submitted were done by Qingqu Guo. Conception and design were also done by Dan Wu.

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Availability of data and materials

The data and materials used and analyzed during our research are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The Ethics Committee of The Second Affiliated Hospital, College of Medicine, Zhejiang University and The First Affiliated Hospital of Zhejiang Chinese Medicine University approved this study, and the patient provided written informed consent form.

Consent for publication

No applicable.

Competing interests

The authors declare that they have no competing of interests.

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Figures

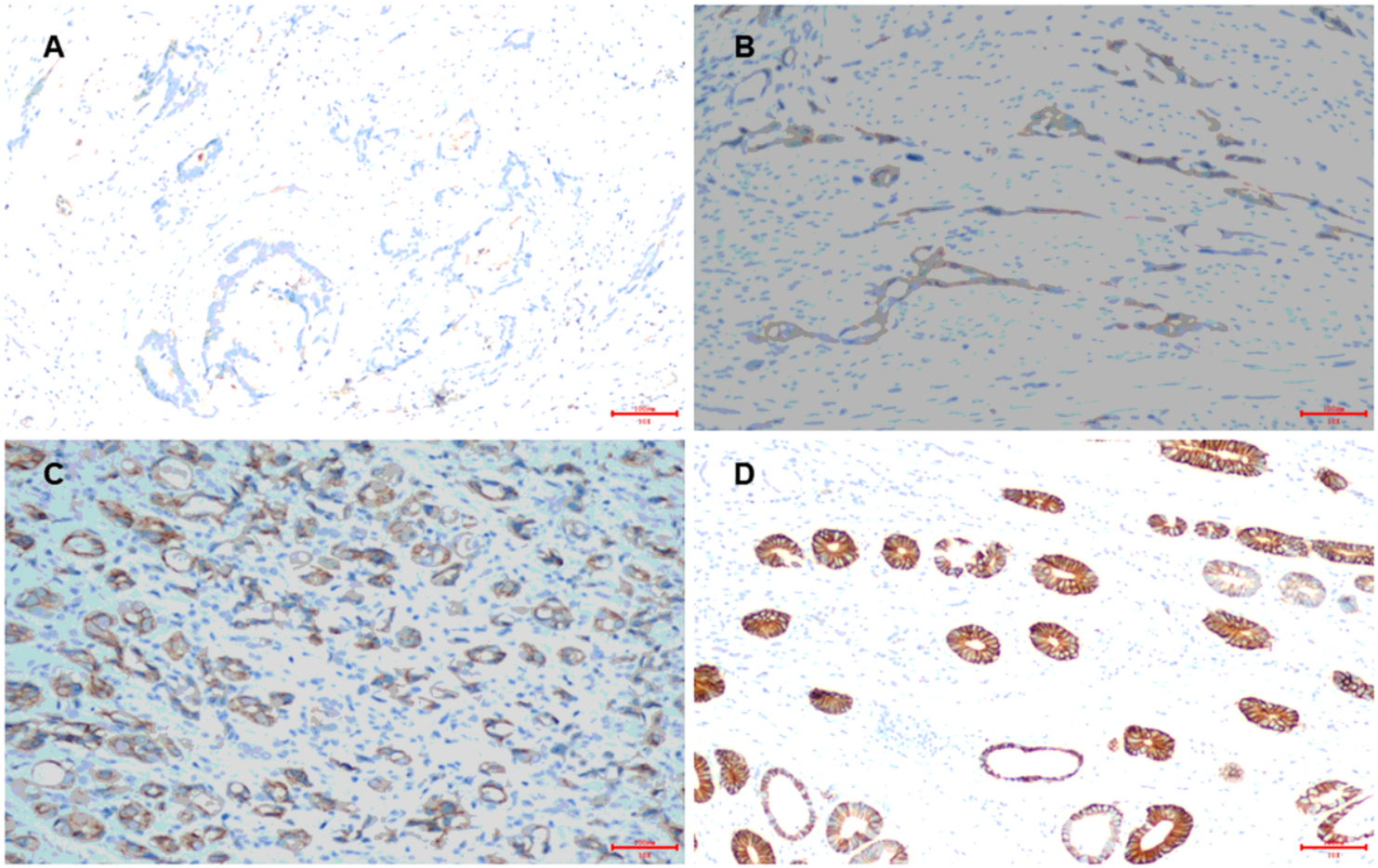

Figure 1

Expression of Her2 protein in the tested samples detected by immunohistochemistry (×10). (A) Immunostaining shows no staining on tumor cell membrane. (B) Immunostaining shows positive reaction (1+). (C) Immunostaining shows positive reaction (2+). (D) Immunostaining shows positive reaction (3+) with complete or basolateral membranous staining.

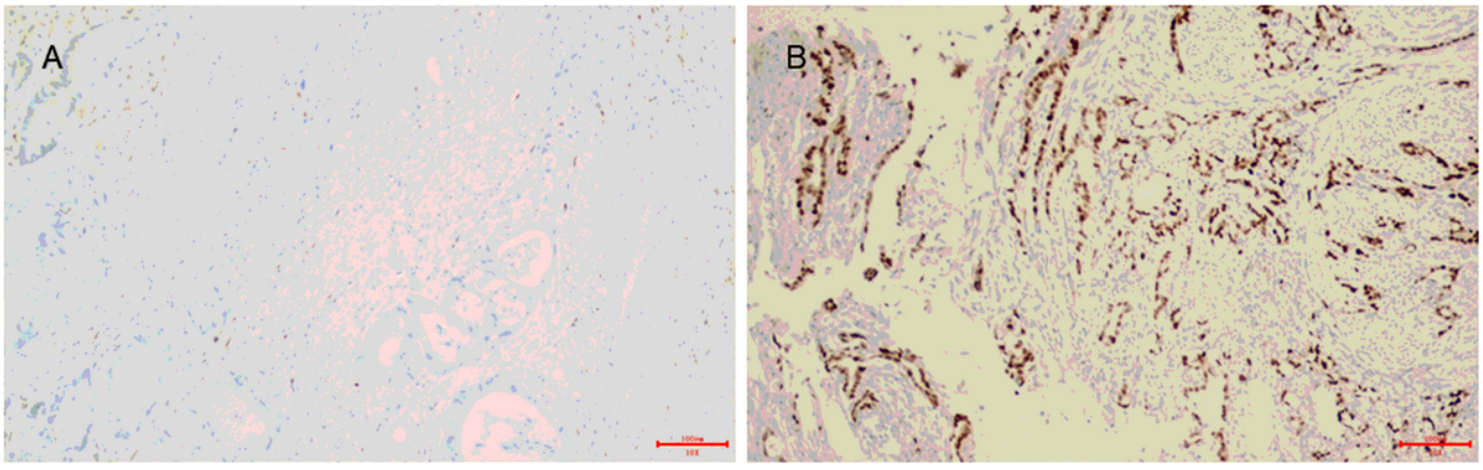

Figure 2

The expression of P53 immunohistochemistry (×10). (A) Negative expression of P53. (B) Positive expression of P53.