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Overexpression of IL-8 and Wnt2 is associated with prognosis of gastric cancer

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Abstract

Introduction. This study is to detect the expression of inflammatory factor or neutrophil-activating factor IL-8 and Wnt2 in gastric cancer (GC) and investigate the involvement of IL-8 and Wnt2 expressions in the clinicopathological indexes and prognosis.

Material and methods. We detected the expression of IL-8 and Wnt2 in 100 GC tissues and 40 normal gastric mucosae using immunohistochemistry. The relationships between the IL-8 and Wnt2 expression and the clinicopathological characteristics were explored. The relationship between IL-8 expression, Wnt2 expression, and prognosis of GC was analyzed by survival curve and survival regression.

Results. The expression of IL-8 and Wnt2 in GC tissue was 64% and 75% respectively, which was significantly higher than that in adjacent normal gastric mucosa tissues, moreover, expressions of IL-8 and Wnt2 were positively correlated. The positive rate of IL-8 and Wnt2 expressions were correlated with lymph node metastasis and TNM staging $P < 0.01$, and Wnt2 was also correlated with infiltration depth ($P = 0.021$), but there was no difference with age, sex, and differentiation ($P > 0.05$). The 3-year survival analysis showed that the survival rates of IL-8- and Wnt2-positive patients were 20% and 24%, respectively, which were significantly lower than those of negative patients. Cox regression analysis showed that IL-8 and Wnt2 may be independent factors affecting the prognosis of GC.

Conclusions. Our data demonstrated that the overexpression of IL-8 and Wnt2 could be isolated prognostic factors in patients with GC and, possibly, may present new targets for the treatment of GC. *(Folia Histochemica et Cytobiologica 2022, Vol. 60, No. 1, 66–73)*

Key words: gastric cancer; interleukin-8; Wnt2; prognosis; IHC

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Introduction

Gastric cancer (GC) is one of the common malignant tumors of the digestive tract, which is the fifth most common malignant tumor (accounting for 6% of all malignant tumors) and the sixth leading cause of cancer death [1]. In 2018, more

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than 1 million people worldwide were diagnosed with GC. In China, GC has the second-highest incidence and its mortality rate ranks only second to lung cancer; and, there are estimated 679 000 new cases and 498 000 deaths annually [2]. About 25% of all malignant tumors worldwide are directly related to infection and chronic inflammation [3]. The persistence of chronic inflammation has been found to be directly involved in carcinogenesis and progression of GC. Especially, the infection with H. pylori, which is a class 1 carcinogen, is closely related to the occurrence of GC. H. pylori continuously releases virulence factors when implanted in gastric mucosal cells and can also induce inflammatory reactions of the host and peripheral immune cells. NF-κB signaling pathway plays an important role in the inflammatory response and can promote the release of interleukin 8 (IL-8) [4]. IL-8/IL-8 receptor (IL-8R) axis activation not only induces and maintains tumor epithelial-mesenchymal transition (EMT) and cancer stem cell (CSC)-like properties [5, 6], but also remodels the tumor microenvironment in order to better promote tumor metastasis [7]. IL-8 is also known as a proinflammatory CXC chemokine. The *IL-8* gene encodes a protein of 99 amino acids that is subsequently processed to yield a signaling competent protein of either 77 amino acids in nonimmune cells or 72 amino acids in monocytes and macrophages [8–10]. IL-8 is not only involved in the inflammatory response, but also may act as an important regulatory factor within the tumor microenvironment [11]. Some researches have shown that IL-8 is involved in cell proliferation, invasion, and metastasis of cancers, such as colon, head, and neck, pancreatic and esophageal cancers [8, 12, 13]. Additionally, the overexpression of IL-8 is reported to be correlated with poor prognosis of numerous solid tumors [11], such as GC [14], melanoma [15], bladder cancer [16], breast cancer [17], and prostate cancer [18].

Wnt signaling pathway is involved in cell differentiation, proliferation, apoptosis, and migration [19]. Studies have shown that excessive activation of the Wnt signaling pathway in GC is very common, which regulates the self-renewal of GC stem cells [20]. The human *WNT2* gene is located at chromosome 7q31, and the human *WNT2B* gene (also known as *WNT13*) is located at chromosome 1p13 [21]. These two genes show 68.6% total amino-acid identity. *WNT2* mRNA is expressed in the human fetal lung and placenta but is almost undetectable in the normal gastrointestinal tract [22]. Some studies have shown that WNT2 is highly expressed in GC and WNT2 is regarded as a tumor marker of GC and colorectal cancer, which would activate the Wnt/β-catenin pathway [23, 24]. *WNT2* gene is one of the targets for pharmacogenomics in the field of oncology [25]. We found in a previous study [26] that the expressions of IL-8 and Wnt2 in GC cells were increased, and there was a positive correlation between these two proteins. However, the mechanism remained obscure. In this study, we found by immunohistochemistry that the expression of IL-8 and Wnt2 were significantly upregulated in GC tissues and the expression of IL-8 was correlated with HMBOX1 in GC. Moreover, the overexpression of these two proteins was significantly associated with clinicopathologic factors and overall survival of patients with GC.

Materials and methods

Clinical GC specimen and clinical information. Pathologically confirmed GC tissue samples were obtained from 100 GC patients after radical total gastrectomy or subtotal gastrectomy from December 2014 to Oct January 2016 in Gansu Provincial Cancer Hospital. The peripheral lymph nodes were routinely dissected in order to determine whether there is lymph node metastasis and to assist in the determination of pathological stages. All the patients did not receive chemotherapy or radiotherapy before the operation. Forty cases of normal gastric mucosa adjacent to cancer were randomly selected as control (distance from cancer tissue > 5 cm). The median age of these 100 patients was 57.01 ± ± 9.45 years (range 29–78 years). There were 47 cases of ≥ 60 years old and 53 cases of < 60 years old. There were 77 males and 23 females. Pathological stages were determined according to American Joint Committee on Cancer (AJCC) 8th Edition TNM staging. Of the 100 patients, 41 cases had early GC (IA-IIB stage) and 59 cases had advanced GC (IIIA–IV stage). According to the degree of pathological differentiation, there were 42 cases in the high and middle differentiation group, and 58 cases in the low differentiation and undifferentiation group. According to the depth of infiltration of cancer cells, 47 cases were with infiltration depth not reaching the serosa layer and 53 cases were with infiltration depth invading the whole gastric wall. According to lymph node metastasis, there were 32 cases without lymph node metastasis. This study received ethical approval from the Ethics Committee of Gansu Provincial Cancer Hospital. Each participant gave written informed consent.

Immunohistochemical analysis. Immunohistochemistry was performed according to a previously described procedure [27]. The paraffin-embedded tissue was sectioned at about 3 μm thickness, dewaxed, and hydrated. Then, antigen retrieval was performed using the pressure cooker by incubating the slides in EDTA buffer (pH 8.0) for 3 min. After rinsing,

Table 1. Positive rates of IL-8 and Wnt2 expressions assessed by immunochemistry in human gastric cancer (GC) and normal gastric mucosa

Group	n	IL-8		P	Wnt2		P
		Positive	Negative		Positive	Negative	
Normal gastric mucosa	40	7 (17.5%)	33 (82.5%)	0.000	5 (12.5%)	35 (87.5%)	0.000
GC tissue	100	64 (64%)	36 (36%)		75 (75%)	25 (25%)	

Pearson chi-square test.

the sections were then incubated with 10% goat serum for 15 min to block nonspecific binding. The samples were incubated at 37°C for 1 h after adding the anti-mouse IL-8 antibody (1:2000 dilution; ImmunoWay, USA) and anti-rabbit Wnt2 antibody (1:600 dilution; Bioss, Beijing, China). Then, after rinsing, incubation with HRP-Polymer anti-Mouse & Rabbit antibody (Zhongshan Jinqiao Biotechnology co., Ltd, Beijing, China) was performed for 1 h. After DAB staining and hematoxylin counterstaining, the sections were observed under an optical microscope. Two pathologists who were blinded to the experimental procedures evaluated the staining intensity and percentage of positive staining. The intensity of the immunostaining was rated on a 0 to 3 scale: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The percentage of positive cells was scored as follows: 0 (< 10% positive cells), 1 (11–25% positive cells), 2 (26–50% positive cells), 3 (51–75% positive cells), and 4 (76–100% positive cells). According to the sum of these two scores, the tumor tissues were classified as IL-8- and Wnt2-positive (score 3–6) or negative (score 0–2).

Statistical analysis. Statistical analysis was performed using SPSS 22.0 software package (IBM Corp., Amrok, NY, USA). $P < 0.05$ was considered statistically significant, and $P < 0.01$ was considered highly statistically significant. Comparison of the categorical data was performed by the χ^2 test. Kendal rank correlation analysis and the Spearman rank correlation test were used for correlation analysis was applied to analyze the correlations of IL-8 expression and Wnt2 with clinicopathological parameters. Kaplan-Meier method was applied to carry out survival analysis, and differences between survival curves were tested by the log-rank test. Cox proportional hazards regression model was used to examine univariate and multivariate analyses.

Results

Overexpression of proinflammatory IL-8 in GC

As shown in Table 1, the positive rate of IL-8 was 64% (64/100) in 100 cases of GC and 17.5% (7/40) in 40 cases of the normal gastric mucosa. The expression of IL-8 in GC was significantly higher than that in normal gastric mucosa ($P < 0.01$). The positive expression of IL-8 in GC tissue and its negative expression in normal gastric mucosa were shown in Fig. 1. IL-8 was highly expressed in the cell membrane and cytoplasm of GC cells.

Overexpression of Wnt2 protein in GC

As shown in Table 1, the positive rate of Wnt2 was 75% (75/100) in 100 cases of GC and 12.5% (5/40) in 40 cases of the normal gastric mucosa. The result indicated that the expression of Wnt2 in GC was significantly higher than that in normal tissues ($P = 0.000$). Fig. 2 displays the positive expression of Wnt2 in GC tissue and its negative expression in normal gastric mucosa. Wnt2 was highly expressed in the cell membrane and cytoplasm of GC cells.

Correlation between IL-8 and Wnt2 expression in GC

The result was shown in Table 2. In 64 cases of IL-8 positive GC, the number of Wnt2 positive cases was 58 (90.6%). The number of Wnt2 positive cases in 36 cases of IL-8 negative GC was 17, *i.e.,* 47.2% ($P = 0.000$). There was a positive correlation between the expression of IL-8 and Wnt2 in GC.

Correlation between IL-8 and Wnt2 with clinicopathological features in patients with GC

The positive rate of IL-8 and Wnt2 expressions and the clinicopathological parameters of GC are presented in Table 3. IL-8 and Wnt2 positive expression were significantly correlated with lymph node metastasis and TNM staging ($P < 0.01$). The positive ratio of these two proteins in early GC (IA-IIB stage) and GC without lymph node metastasis were less than advanced GC (IIIA–IV stage) and GC with lymph node metastasis. For the depth of infiltration, the expression of IL-8 showed no significant difference. However, Wnt2 in GC with invasion into serosa was higher than that of cases without serosa invasion ($P = 0.021$). Moreover, there was no significant difference in IL-8 and Wnt2 expressions between patients with different ages, sex and tumor differentiation ($P > 0.05$).

IL-8 and Wnt2 overexpression predicts poor prognosis in GC

The 100 cases of GC patients were with complete follow-up data and were followed up for 3 to

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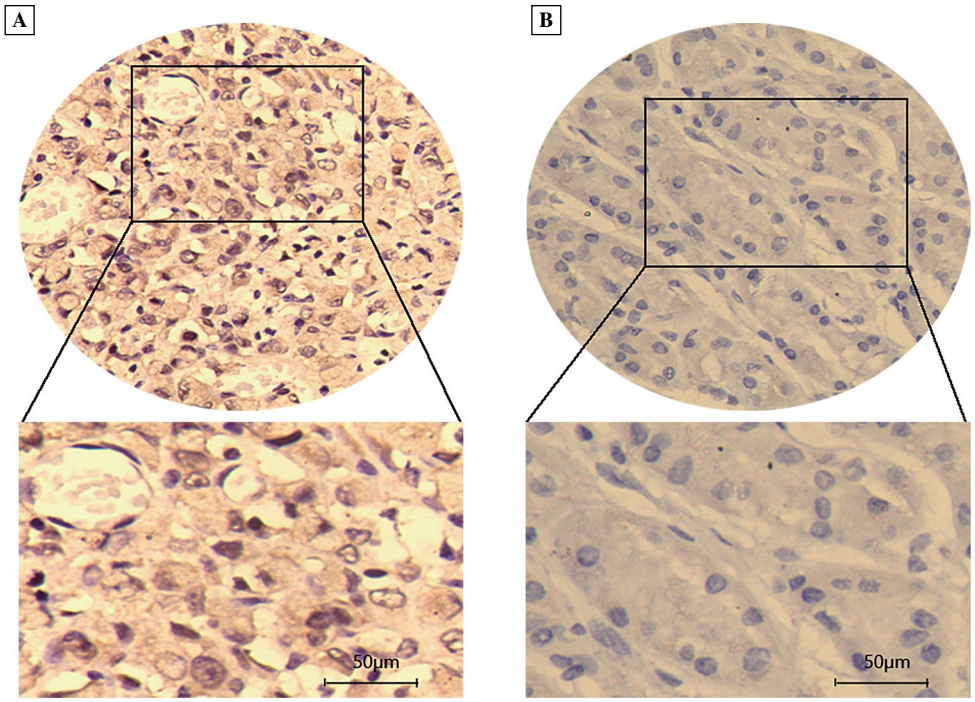

Figure 1. Representative images of interleukin 8 (IL-8) expression were assessed by immunochemistry in gastric cancer and normal gastric mucosa detected by immunohistochemistry. **A.** High IL-8 expression in the cytoplasm of gastric cancer cells. **B.** Negative IL-8 expression in normal gastric mucosa. Magnification: 200×.

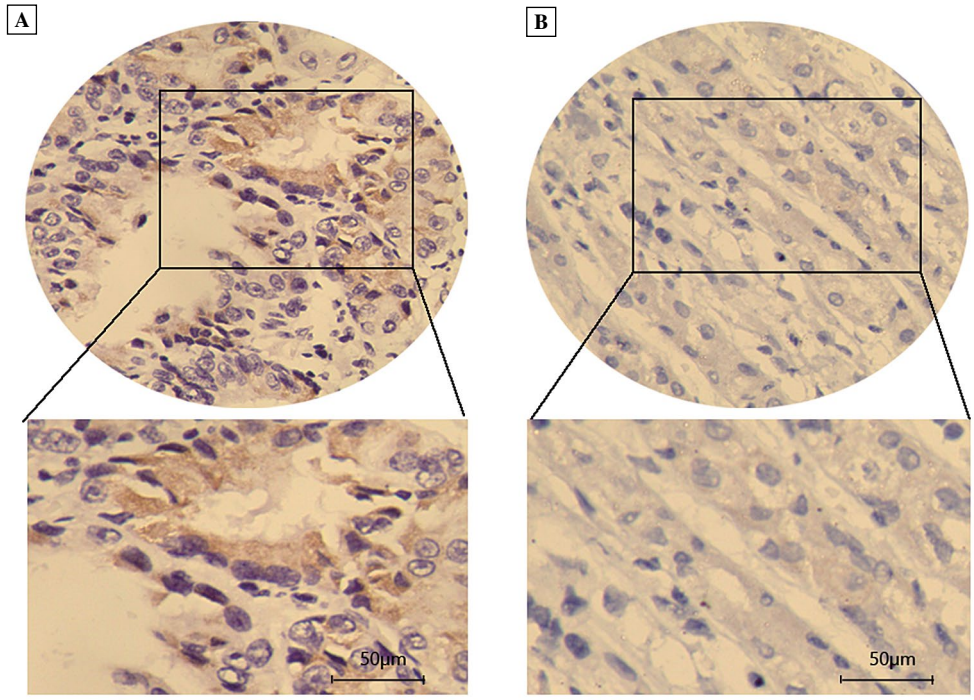

Figure 2. Representative images of Wnt2 expression assessed by immunochemistry in gastric cancer and normal gastric mucosa detected by immunohistochemistry. **A.** High Wnt2 expression in the cytoplasm of gastric cancer cells. **B.** Negative Wnt2 expression in normal gastric mucosa. Magnification: 200×.

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Table 2. Correlation between expression of IL-8 and Wnt2 assessed by immunochemistry in gastric cancer

IL-8	Wnt2		Total, n	Correlation coefficient	*P*
	Positive	Negative			
Positive	58	6	64	0.481	0.000
Negative	17	19	36		
Total, n	75	25			

Kendal rank correlation analysis was used to test, tau_b = 0.481, P = 0.000, which showed that there was a positive correlation between the expression of IL-8 and Wnt2 in GC.

Table 3. Correlations of IL-8 and Wnt2 expression assessed by immunochemistry with clinicopathologic parameters of gastric cancer

Parameters	n	IL-8		r	P	Wnt2		r	P
		Positive	Negative			Positive	Negative		
Gender									
Female	23	15	8	–0.014	0.89	18	5	–0.041	0.682
Male	77	49	28			57	20		
Age (y)									
< 60	53	31	22	–0.122	0.225	39	14	–0.035	0.73
≥ 60	47	33	14			36	11		
Differentiation									
Well/Moderate	55	33	22	–0.092	0.359	38	17	–0.151	0.133
Poor/Undifferentiated	45	31	14			37	8		
TNM stage									
I/II	42	18	24	–0.375	0.000	17	25	–0.623	0.000
III/IV	58	46	12			56	2		
Lymph node metastasis									
No	23	15	18	–0.271	0.007	17	16	–0.36	0.000
Yes	67	49	18			57	10		
Depth of invasion									
Without serosa	48	28	20	–0.37	0.47	31	17	–0.231	0.021
Into serosa	52	34	18			44	8		

36 months. The median overall survival was 27 months. The Kaplan-Meier survival analysis and Log Rank test revealed that the survival times of the IL-8- and Wnt2-positive groups were significantly lower than those of the negative group (Fig. 3). The average survival time of IL-8-positive patients was 20.1 months. The 1-year, 2-year, and 3-year survival rates were 70%, 40%, and 20%, respectively. However, the average survival time of the IL-8-negative patients was 31.6 months. The 1-year, 2-year, and 3-year survival rates were 100%, 82%, and 65%, respectively (χ^2 = 25.09, P = 0.000). Thus, the survival rate of IL-8-positive patients was significantly lower than that of negative patients. The average survival time of Wnt2 positive patients was 21.8 months and the 1-year, 2-year, and 3-year survival rates were 78%, 45%, and 24%; whereas, the patients with negative Wnt2 immunoexpression had an average survival time of 31.9 months and their 1-year, 2-year, and 3-year survival rates were 96%, 88%, and 69% (χ^2 = 15.31, P = 0.000). Therefore, the survival rate of Wnt2-positive patients was significantly lower than that of Wnt2-negative patients.

Cox regression analysis of survival and multiple factors

In order to determine the relationship among survival time, age, differentiation, depth of invasion, lymph

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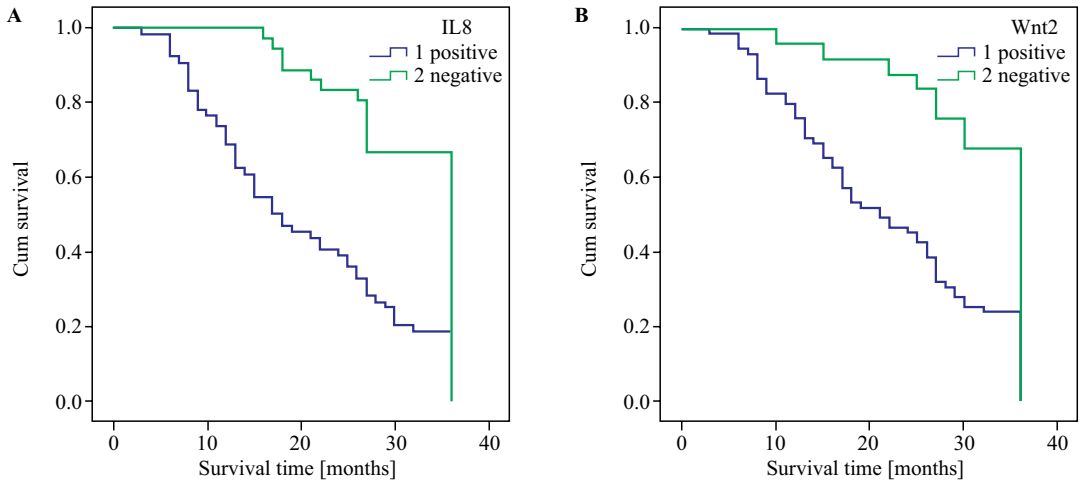

Figure 3. The expression of IL-8 and Wnt2 was assessed by immunochemistry in gastric cancer tissue and survival analysis. **A.** IL-8 expression and survival analysis: the survival rate of IL-8-positive patients was significantly lower than that of IL-8-negative patients ($P = 0.000$). **B.** Wnt2 and survival analysis: the survival rate of Wnt2-positive patients was significantly lower than that of Wnt2-negative patients ($P = 0.000$).

Table 4. Multivariate analysis of prognostic factors in gastric cancer patients

	Non standardized coefficient		Standardized coefficient	T	P
	B	Standard error	Beta		
(constant)	48.310	2.401		20.123	0.000
Age	–3.054	1.476	–0.140	–2.069	0.041
Differentiation	–0.599	1.531	–0.027	–0.391	0.696
Depth	0.076	1.644	0.003	0.046	0.963
Lymph node metastasis	–0.870	2.219	–0.037	–0.392	0.696
Stage	–6.021	1.417	–0.490	–4.249	0.000
IL-8	–4.021	1.757	–0.177	–2.289	0.024
Wnt2	–5.169	2.148	–0.205	–2.407	0.018

node metastasis, stage, IL-8, and Wnt2, the data of 100 patients were introduced into the multiple linear regression equation and the results of the analysis were shown in Table 4. The differentiation of tumor, depth of invasion, and lymph node metastasis were not independent factors affecting the prognosis of GC ($P > 0.05$). However, age, stage, IL-8, and Wnt2 had a linear regression relationship with survival time ($P < 0.05$), and they were independent factors affecting the prognosis of GC. The standardized coefficients of age, stage, IL-8, and Wnt2 were all less than 0, indicating that the older the patients were, the later the stage was the shorter was the survival time of the patients. The survival time of IL-8- and Wnt2-positive GC patients was shorter than that of negative GC patients.

Discussion

In the 1980s, Peveri *et al.* found that bacterial lipopolysaccharide would stimulate blood monocytes to produce a kind of secretory protein, which had the ability to recruit and activate neutrophils to migrate to inflamed tissues and trigger the inflammatory reaction of local diseases of the body. Therefore, this secretory protein or chemokine was called a neutrophil-activating factor [28]. In 1989, a new neutrophil-activating protein was found by Larsen *et al.*, which had T cell and neutrophil chemotactic and activating functions. This cytokine was termed interleukin-8 (IL-8) [29]. Subsequently, it has been shown that many cell types other than monocytes, including blood monocytes,

alveolar macrophages, fibroblasts, endothelial cells, and epithelial cells, can produce large quantities of IL-8 or *IL-8* mRNA, in response to stimulation with endotoxin, IL-l, or tumor necrosis factor [8, 30]. The functional study revealed that IL-8 overexpressed on some tumor cells, and that tumor-derived IL-8 through autocrine and paracrine could alter the composition of immune infiltrates in the tumor microenvironment and induce angiogenesis and growth of the cancer cells [31], which even involved in EMT and CSC-like change. However, whether IL-8 can be used as a prognostic molecular marker for GC is not determined. A meta-analysis of 80 studies (1843 patients) showed that high IL-8 expression could be a negative prognostic biomarker for patients with GC[32]. In this study, we found that the expression rate of IL-8 in GC was 64%, which was significantly higher than that in normal gastric tissue (17.5%). The positive rate of IL-8 in advanced GC and GC with lymph node metastasis was obviously higher than that in early GC and GC without lymph node metastasis. The survival rate of patients with positive expression of IL-8 was significantly lower than that of those with negative expression.

In our previous study, we found that there had a positive correlation between the secretion of many IL-8 and the high expression of Wnt2 in induced malignant immortalized human gastric epithelial cells (GES-1 cells). IL-8 might be involved in activating the expression of Wnt2. The results have not yet been published. Wnt2 is the upstream protein of the Wnt/β-catenin signaling pathway, which helps the activation of the signaling pathway and the regulation of downstream target genes, and ultimately promotes EMT and CSC-like changes in GC cells. It has been shown that Wnt2 can be used as a tumor marker of GC. Subsequently, we found that the positive expression rate of Wnt2 in 100 cases of GC was 75%, which was significantly higher than that in normal gastric tissues (12.5%). The positive rate of Wnt2 in advanced GC, with lymph node metastasis and full-thickness invasion of GC, was obviously higher than that in early-stage, with no lymph node metastasis and non-invasion to serous layer. The survival rate of patients with positive expression of Wnt2 was significantly lower than that of those with negative expression. We also found a positive correlation between the expression of IL-8 and Wnt2 in GC. Cox regression analysis further showed that IL-8 and Wnt2 were independent factors affecting the prognosis of GC and could be used as new targets for the treatment of GC.

Overall, IL-8 and Wnt2 expressions are upregulated in patients with GC, and they have a positive correlation. Additionally, IL-8 and Wnt2 are independent factors affecting survival time in patients with GC. These findings also confirm that IL-8 and Wnt2 could be regarded as candidate prognostic factors after treatment in patients with GC.

Conclusion

Overall, we found that IL-8 and Wnt2 expressions are upregulated in patients with GC, and they have a positive correlation. Moreover, we showed that IL-8 and Wnt2 are isolated factors regarding survival time in patients with GC. These findings also confirmed that IL-8 and Wnt2 could be regarded as candidate prognostic factors after treatment in patients with GC.

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Ethics approval and consent to participate

All experimental procedures were approved by the Ethics Committee of Gansu Provincial Cancer Hospital.

Competing interests

The authors declare no potential conflicts of interest.

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