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Expression and significance of IGF-2, PCNA, MMP-7, and α-actin in gastric carcinoma with Lauren classification

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Background/aims: *By detecting the expression and distribution of insulin-like growth factor-2, proliferating cell nuclear antigen, and matrix metalloproteinase-7 in cancer cells and the expression of α-actin in interstitial myofibroblasts, we studied their differences and their relationship in intestinal type and diffuse type gastric cancer with Lauren classification.* ***Materials and Methods:*** *Clinical and pathological data of 50 patients with gastric adenocarcinoma who underwent primary surgical resection between 2003 and 2008 in Qianfoshan Hospital were collected. The cancer was classified as intestinal or diffuse type with Lauren classification. Immunohistochemical technique was used to detect the protein expression of insulin-like growth factor-2, proliferating cell nuclear antigen, matrix metalloproteinase-7, and α-actin in both gastric cancer tissue and normal gastric mucosa.* ***Results:*** *The expression of insulin-like growth factor-2, proliferating cell nuclear antigen, matrix metalloproteinase-7, and α-actin in the gastric tissue was significantly higher than in the normal gastric mucosa. Insulin-like growth factor-2 was mainly expressed in the nucleus in diffuse gastric cancer and in the cytoplasm in intestinal type. The expression of insulin-like growth factor-2 in the nucleus was correlated with depth of tumor invasion, lymph node metastasis and staging. Proliferating cell nuclear antigen and matrix metalloproteinase-7 showed higher expression in diffuse than in intestinal type gastric cancer. The overexpression of proliferating cell nuclear antigen was relevant to lymph node metastasis in gastric cancer. The overexpression of matrix metalloproteinase-7 was relevant to invasion, lymph node metastasis, distant metastasis, and staging in gastric cancer. There was no significant difference in α-actin expression between the intestinal type and diffuse type gastric cancer. The overexpression of α-actin was relevant to cancer invasion and lymph node metastasis.* ***Conclusions:*** *The expression patterns of insulin-like growth factor-2 and the expression intensity of proliferating cell nuclear antigen and matrix metalloproteinase-7 were significantly different between diffuse type and intestinal type gastric cancer cells, but the expression pattern and intensity of the interstitial myofibroblast marker showed no significant difference. The clinical pathology distinction between intestinal type and diffuse type gastric cancer may be due mainly to the change in the genetic structure and the phenotype of epithelial cells, and interstitial myofibroblasts and cancer cells jointly promote the invasion and metastasis of gastric cancer.*

Key words: Gastric carcinoma, Lauren classification, insulin-like growth factor-2, proliferating cell nuclear antigen, matrix metalloproteinase-7, α-actin

Gastrik adenokarsinomda Lauren sınıflamasına göre IGF-2, PCNA, MMP-7 ve α-aktin ekspresyonu ve önemi

Amaç: *Kanser hücrelerinde insülin-benzeri büyüme faktörü-2, prolifere edici hücre nükleer antijeni, matriks metalloproteinaz-7'nin ekspresyon ve dağılımını ve interstisyel miyofibroblastlarda α-aktin ekspresyonunu tespit ederek Lauren sınıflamasına göre intestinal ve yaygın tip gastrik kanserdeki farklarını çalıştık.* ***Gereç ve Yöntem:*** *Qianfoshan Hastanesinde 2003 ile 2008 yılları arasında primer cerrahi rezeksiyon geçiren gastrik adenokarsinomalı 50 hastanın klinik ve patolojik verileri toplandı. Kanser, Lauren sınıflamasına göre intestinal ve yaygın tip olarak sınıflandı. Hem gastrik kanser dokusu hem de normal gastrik mukozada insülin-benzeri büyüme faktörü-2, prolifere edici hücre nükleer antijeni, matriks metalloproteinaz-7 ve α-aktin protein ekspresyonunu tespit etmek için immunohistokimyasal teknik kullanıldı.* ***Bulgular:*** *Gastrik dokuda insülin-benzeri büyüme faktörü-2, prolifere edici hücre nükleer antijeni, matriks metalloproteinaz-7, α-aktin ekspresyonu, normal gastrik mukozadan anlamlı derecede yüksekti. İnsülin-benzeri büyüme faktörü-2 esasen yaygın gastrik kanserin nükleusunda ve intestinal tipin sitoplazmasında eksprese oluyordu.*

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Nukleusta insülin-benzeri büyüme faktörü-2 ekspresyonu, tümör invazyonunun derinliği, lenf nodu metastazı ve evre ile ilişkili idi. Prolifere edici hücre nükleer antijeni, matriks metalloproteinaz-7 yaygın gastrik kanserde intestinal gastrik kansere göre daha fazla eksprese oluyordu. Prolifere edici hücre nükleer antijeninin fazla ekspresyonu gastrik kanserin lenf nodu metastazı ile ilişkili idi. matriks metalloproteinaz-7'nin aşırı ekspresyonu, invazyon, lenf nodu metastazı, uzak metastaz ve gastrik kanser evresi ile ilişkili idi. İntestinal tip gastrik kanser ile yaygın tip arasında α-aktin ekspresyonu açısından anlamlı fark yoktu. α-aktin'in aşırı ekspresyonu kanser invazyonu ve lenf nodu metastazı ile ilişkili idi. ***Sonuç:*** *Yaygın tip ve intestinal tip gastrik kanserde insülin-benzeri büyüme faktörü-2'nin ekspresyon paterni ve prolifere edici hücre nükleer antijeni ile matriks metalloproteinaz-7'nin ekspresyon yoğunluğu anlamlı şekilde farklıydı, ancak interstisyel miyofibroblast belirtecinin ekspresyon patern ve yoğunluğunun farkı anlamlı değildi. İntestinal ve yaygın tip gastrik kanser arasındaki klinik patolojik farklılık, epitel hücrelerinin genetik yapısı ve fenotipindeki değişikliklerdir. İnterstisyel miyofibroblastlar ve kanser hücreleri gastik kanserin invazyon ve metastazı için birlikte artırıcı etki yapmaktadırlar.*

Anahtar kelimeler: Gastrik karsinom, Lauren sınıflaması, insülin-benzeri büyüme faktörü-2, prolifere edici hücre nükleer antijeni, matriks metalloproteinaz-7, α-aktin

INTRODUCTION

According to the origin of gastric cancer, it is divided into intestinal type and diffuse type by the Lauren classification. The intestinal type, deriving from the glandular epithelium of intestinal metaplasia, is mostly well-differentiated and carries a good prognosis, while the diffuse type, originating from the gastric proper glandular epithelium, is poorly differentiated and more prone to invade and metastasize. For a long time, the occurrence of epithelial tumors was attributed to the change in genetic structure and phenotype of epithelial cells, but in recent years, it was found that tumor stroma also played an essential role in the tumorigenesis, differentiation and progression (1). The clinical pathologic differences between intestinal and diffuse type gastric cancer may result from molecular differences in the epithelial and stromal cells.

Insulin-like growth factor-2 (IGF-2) is a mitogenic cytokine with a strong proliferative activity. It is considered to be the important autocrine growth factor in many kinds of tumor cells, and it has already been proven that the overexpression of IGF-2 can promote proliferation and malignant transformation of gastric cancer cells (2). However, the relationship between IGF-2 expression and gastric cancer according to Lauren classification has been reported rarely. Proliferating cell nuclear antigen (PCNA) is the auxiliary protein of DNA polymerase δ and plays an important role in DNA replication. It can be used as a good indicator of the gastric cancer cell proliferation and prognosis (3). The extracellular matrix, composed of stromal cells and basement membrane, is the natural barrier to hold back the invasion and metastasis of tumor cells. The matrix metalloproteinases (MMPs) family, which can degrade the extracellular matrix, is the most important group of proteolytic enzymes and is mostly produced by the interstitial cells. MMP-7 is relatively unique because it is mainly produced in epithelial cells (4). In the past, research about the relationship between MMP-7 expression and Lauren classification in gastric cancer has been quite inconsistent. Some researchers found that MMP-7 showed higher expression in intestinal type gastric cancer than in the diffuse type (5,6). Kitoh et al. (7) observed the opposite result. Recent studies have suggested that MMP-7 expression has nothing to do with the Lauren classification (8). Tumor interstitial myofibroblasts, also known as tumor-associated myofibroblasts, represent the most important class of cells in the stroma. α-actin is one of the most commonly used markers of myofibroblasts (9). Studies about colon, liver, pancreas, prostate, and other cancers have found that cancer stroma myofibroblasts may promote tumor invasion and metastasis (10-14), while the research on gastric cancer myofibroblasts is rare.

We used immunohistochemical technique to detect the protein expression of IGF-2, PCNA, MMP-7, α-actin in intestinal type and diffuse type gastric cancer, and their expression patterns in stromal cells and cancerous cells were analyzed to discuss the relationship with the Lauren classification.

MATERIALS AND METHODS

Clinical and Pathological Data

Surgical specimens of 50 cases of gastric cancer were collected from January 2009 to December 2010 in Qianfoshan Hospital Affiliated Hospital to Shandong University. According to Lauren classification, 23 cases (9 males, 14 females; age range,

40-70 years; mean age, 55±10.7 years) had diffuse type gastric cancer, and this group included 3 cases of moderately differentiated tubular adenocarcinoma, 7 cases of poorly differentiated adenocarcinoma, and 13 cases of signet-ring cell carcinoma. Twenty-seven cases (17 males, 10 females; age range, 43-72 years; mean age, 58±8.3 years) had intestinal type gastric cancer, and this group included papillary adenocarcinoma in 7 cases, highly and moderately differentiated tubular adenocarcinoma in 14 cases, and poorly differentiated adenocarcinoma in 6 cases. There were 21 normal controls (13 males, 8 females; age range, 39-60 years; mean age, 54±8.4 years). All pathology specimens were diagnosed and reviewed by three veteran pathologists.

According to the TNM stage of the Union for International Cancer Control (UICC) in 1997, gastric cancer is divided into stages Ia, Ib, II, IIIa, IIIb, or IV. Twenty-seven cases with stages Ia and Ib were considered as the early clinical gastric cancer (stage I), including 17 male and 10 female patients, with an average age of 56±9.4 years. Twenty-three cases of stage II-IV gastric cancer were merged in the late stage gastric cancer (stages II-IV), and included 10 males and 13 females, with a mean age of 57±9.8 years. On the basis of the depth of cancer invasion, 20 cases were limited to mucosa and submucosa (T1) and 30 cases broke through the submucosa (T2-4). In accordance with metastasis of gastric cancer, it can be divided into 24 cases with lymph node metastasis and 26 cases without lymph node metastasis, or 10 patients with distant metastasis and 40 patients with no distant metastasis. No statistically significant differences in age and gender existed between the groups of patients.

Methods

Specimens were fixed by 10% paraformaldehyde, conventionally dehydrated, embedded in paraffin, and sliced in 4μm serial sections. Microwave EDTA (pH 9.0) antigen retrieval method was used. Two-step immunohistochemical method was used. Rabbit polyclonal anti-human IGF-2 antibody (Abcam, ab9574, dilution 1:100), mouse anti-human PCNA monoclonal antibody (Abcam, ab29, dilution 1:1000), and mouse monoclonal anti-human MMP-7 antibody (Abcam, ab3205, dilution 1:80) were all purchased from Abcam company, and mouse monoclonal anti-human α-actin antibody (Santa Cruz, sc58669, dilution: 1:300) was purchased from Santa Cruz company.

Results Guidelines

1. Determination of positive cells: IGF-2 was considered positive when the cytoplasm or nucleus had evenly stained brown granules. For MMP-7, buffy staining of the cell membrane or cytoplasm was considered as positive staining. PCNA protein was assessed by the criterion of brown granular appearance in the nucleus. α-Actin staining was considered positive when brown or brown-yellow granules with clear background appeared in the cytoplasm.

2. Determination of staining intensity: The overall staining of the slices was observed by 400 times magnification. Five representative regions were selected to calculate 200 cells each. IGF-2, MMP-7 and α-actin staining intensity levels: non-color or staining in less than 10% of cells was considered negative (-), color tinting or staining in 10%-30% of cells was considered weakly positive (+), moderate staining or colored staining in 31%-50% of cells was considered positive (++), and staining in more than 50% of cells was considered strongly positive (+++). PCNA staining intensity levels: non-color or less than 25% of stained cells was considered negative (-), color tinting or staining in 25%-50% of cells was considered weakly positive (+), moderate staining or colored staining in 51-75% of cells was considered positive (++), and staining in more than 75% of cells was considered strongly positive (+++).

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) 13.0 software. Measurement data were analyzed using the Student's t test, while categorical data were studied using chi-square test or Fisher exact test. Statistical significance was accepted as $p<0.05$.

RESULTS

Comparison of the Expression of IGF-2, PCNA, MMP-7, and α-Actin in Normal Gastric Mucosa and Gastric Cancer Tissue

IGF-2 was rarely expressed in the stroma. IGF-2 was expressed in epithelial cells in the normal gastric mucosa. IGF-2 was expressed in the nucleus or cytoplasm of cancer cells in gastric cancer tissues, and its expression level was significantly higher than in normal mucosa ($\chi^2=39.13$, $p<0.01$). PCNA was mainly expressed in epithelial cells and nuclei of cancer cells, and its expression was

significantly higher in gastric carcinoma than in the normal gastric mucosa (χ^2=71.00, p<0.01). MMP-7 had no expression in normal mucosa and rare expression in gastric cancer stroma; however, it was expressed in the cytoplasm of cancer cells, with mostly positive expression (χ^2=38.48, p<0.01). α-actin was expressed in the cytoplasm of stromal cells, and its expression was significantly higher in gastric carcinoma than in the normal gastric mucosa (χ^2=25.91, p<0.01) (Table 1).

Comparison of Expression of IGF-2, PCNA, MMP-7, α-Actin in Intestinal and Diffuse Type Gastric Cancer

IGF-2 was expressed in gastric cancer cell nucleus or cytoplasm, and in a minority (6 patients), it was expressed in both cancer cells plasma and nucleus. The expression intensity of IGF-2 between diffuse gastric cancer and intestinal cancer was not significantly different (χ^2=1.05, p>0.05) (Table 2). According to the main expression area of IGF-2, specimens can be divided into two groups as nucleus expression in 24 cases and cytoplasmic expression in 26 cases. IGF-2 nuclear expression was seen most frequently in diffuse gastric cancer (21/23) (Figure 1A), while the majority of IGF-2 expression in cytoplasm was seen in intestinal type gastric cancer (24/27) (Figure 1B). There were significant differences between the two types of gastric cancers (χ^2=32.00, p<0.01). MMP-7 showed higher expression in the diffuse type gastric cancer than in the intestinal type (Figure 2A, B) (χ^2=19.54, p<0.01). PCNA showed higher expression in diffuse type than in intestinal type gastric cancer (Figure 3A, B) (χ^2=6.52, p<0.05). The expression of α-actin showed no significant difference between diffuse type and intestinal type gastric cancer (Figure 4A, B) (χ^2=2.98, p>0.05), and was distributed irregularly among cancer cell nests in small pieces, mass or cord-like patterns.

Relationship Between IGF-2 Expression Patterns and Invasion and Metastasis of Gastric Cancer

Gastric cancer with IGF-2 expression in the nucleus had deeper infiltration (χ^2=4.33, p<0.05) and more lymph node metastasis (χ^2=9.64, p<0.01) than with IGF-2 expression in the cytoplasm. There was no statistically significant difference in the

Table 1. The expression intensity of IGF-2, PCNA, MMP-7, and α-actin in normal gastric mucosa and gastric cancer

Variable	Normal gastric mucosa (n)				Gastric cancer (n)			
	-	+	++	+++	-	+	++	+++
IGF-2*	0	20	1	0	0	8	12	30
PCNA*	16	5	0	0	0	0	10	40
MMP-7*	21	0	0	0	10	15	15	10
α-Actin*	4	7	0	0	3	14	24	9

*Significantly different compared to normal gastric mucosa (p<0.01).

Table 2. The expression intensity of IGF-2, PCNA, MMP-7, and α-actin in intestinal and diffuse type gastric cancer

Variable	Intestinal type				Diffuse type			
	-	+	++	+++	-	+	++	+++
IGF-2*	0	3	7	17	0	5	5	13
PCNA*	0	0	9	18	0	0	1	22
MMP-7*	7	13	7	0	3	2	8	10
α-Actin	3	7	13	4	0	7	11	5

*Significantly different compared to normal gastric mucosa.

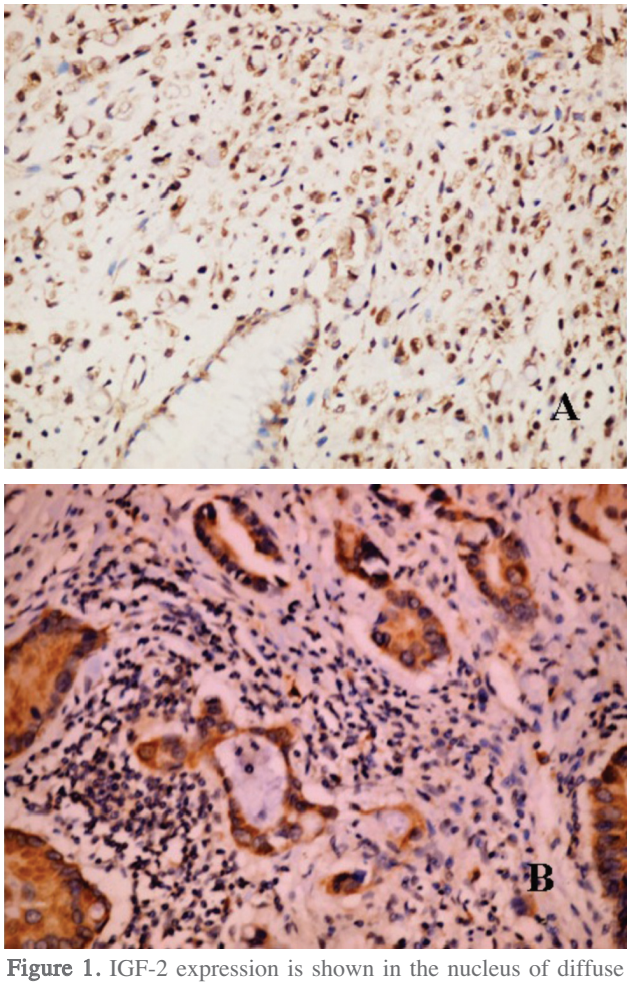

Figure 1. IGF-2 expression is shown in the nucleus of diffuse type gastric cancer (A), and in the cytoplasm of intestinal type gastric cancer (B) (400X).

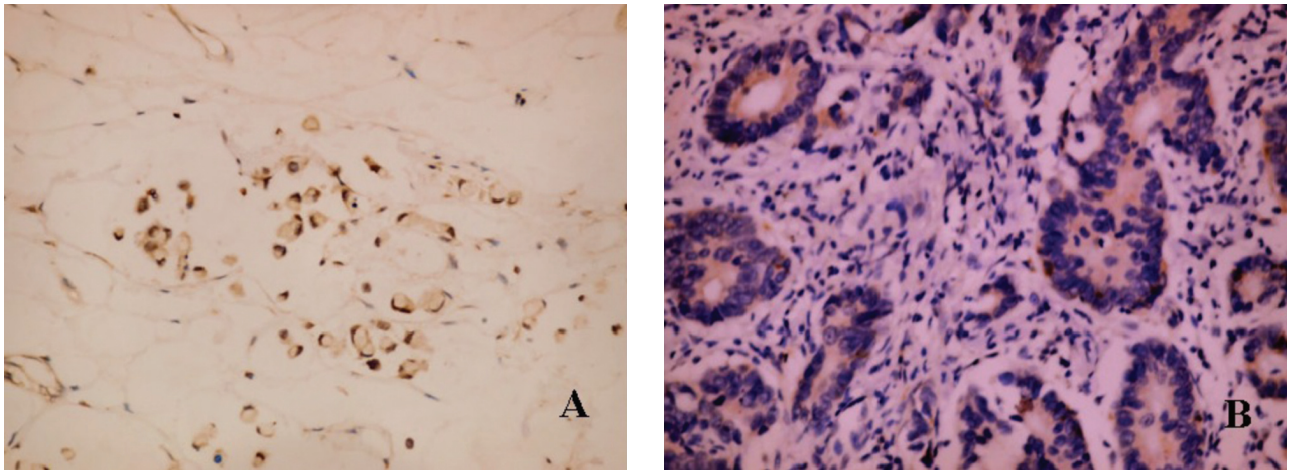

Figure 2. MMP-7 expression is higher in diffuse type gastric cancer **(A)** than in intestinal type gastric cancer **(B)** (400X).

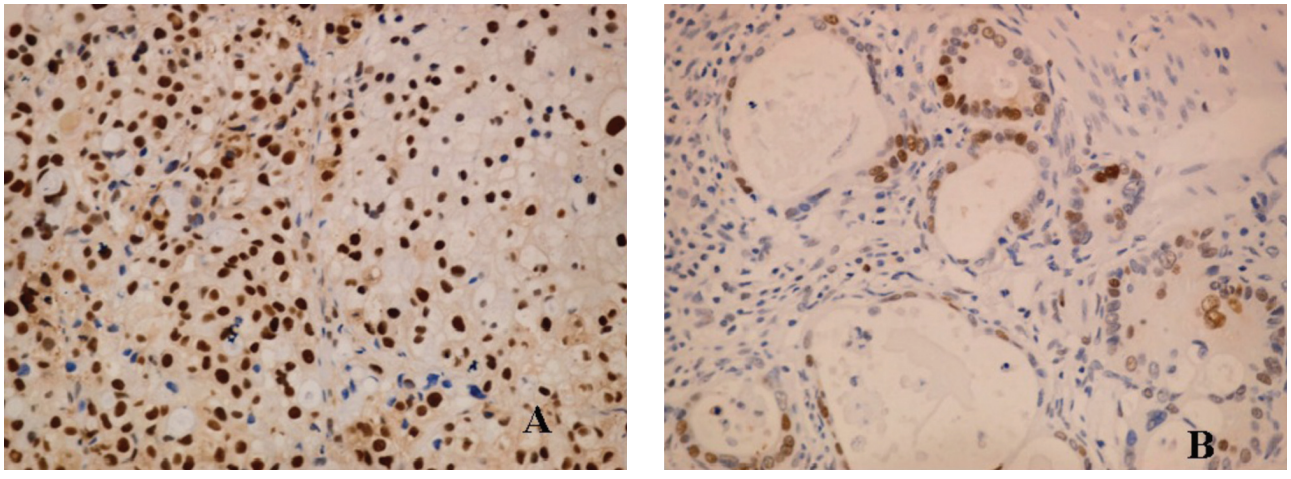

Figure 3. PCNA expression is higher in diffuse type gastric cancer (A) than in intestinal type gastric cancer (B) (400X).

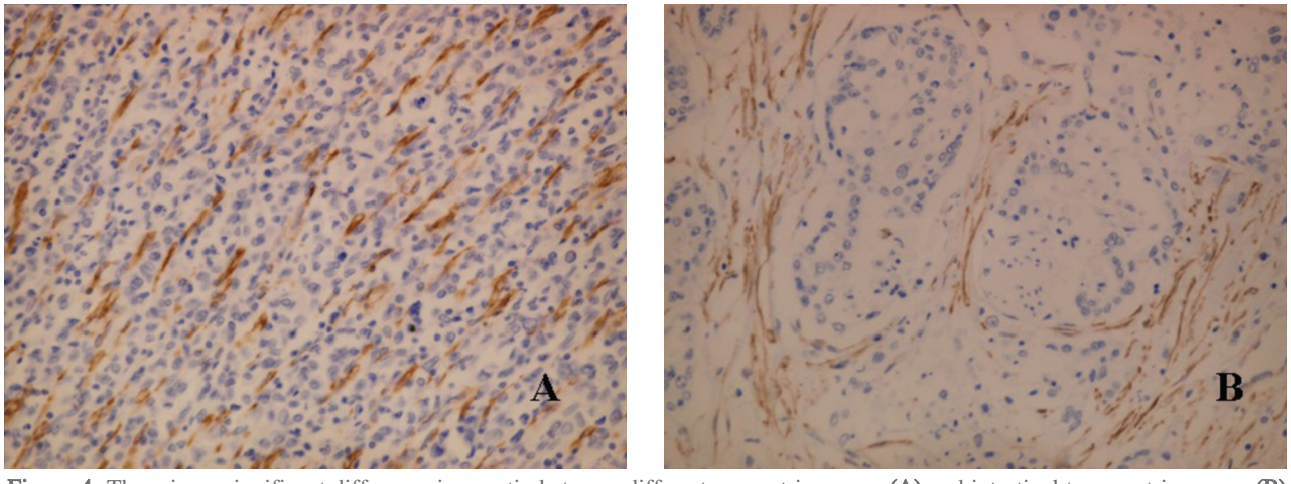

Figure 4. There is no significant difference in α-actin between diffuse type gastric cancer **(A)** and intestinal type gastric cancer **(B)** (400X).

distant metastasis between cases with IGF-2 expression in gastric cancer cell nuclei or in plasma (χ^2=2.42, p>0.05). Compared to those with IGF-2 expression in the cytoplasm, gastric cancer with IGF-2 expression in the nucleus had later staging (χ^2=11.46, p<0.01) (Table 3).

Table 3. The relationship between IGF-2 expression pattern and clinical pathologic parameters in gastric cancer

Variable	Group	Number	IGF-2 expression pattern	
			Nucleus expression	Cytoplasm expression
Infiltration depth*	≤T1	20	6	14
	T2-4	30	18	12
Lymph node metastasis*	No	26	7	19
	Yes	24	17	7
Distant metastasis	No	40	17	23
	Yes	10	7	3
TNM staging*	Stage I	27	7	20
	Stage II-IV	23	17	6

There was a significant difference between the different IGF-2 expression patterns.

Table 4. The relationship between PCNA expression and clinical pathologic parameters in gastric cancer

Variable	Group	Number	PCNA staining intensity			
			-	+	++	+++
Infiltration depth	≤T1	20	0	0	6	14
	T2-4	30	0	0	4	26
Lymph node metastasis*	No	26	0	0	9	17
	Yes	24	0	0	1	23
Distant metastasis*	No	40	0	0	10	30
	Yes	10	0	0	0	10
TNM staging	Stage I	27	0	0	8	19
	Stage II-IV	23	0	0	2	21

*There was a significant difference between PCNA expression and clinical pathologic parameters.

Relationship Between PCNA Expression and Invasion and Metastasis of Gastric Cancer

No significant difference existed in PCNA expression between groups with tumor invasion depth of ≤T1 versus T2-4 (χ^2=2.08, p>0.05). PCNA had higher expression in gastric cancer with lymph node metastasis than without lymph node metastasis (χ^2=7.23, p<0.01). There were no significant differences in PCNA expression between gastric cancer without and with distant metastasis (χ^2=3.13, p>0.05). Similarly, there was no significant difference in PCNA expression between the stage I gastric cancer group and the stages II-IV group (χ^2=3.40, p>0.05) (Table 4).

Relationship Between MMP-7 Expression and Invasion and Metastasis of Gastric Cancer

MMP-7 expression was significantly higher in the group with tumor invasion depth of T2-4 than in the ≤T1 group (χ^2=18.33, p<0.01). MMP-7 expression was increased in gastric cancer with than without lymph node metastasis (χ^2=9.94, p<0.05). MMP-7 expression was also heightened in the gastric cancer with than without distant metastases (χ^2=7.92, p<0.05), and MMP-7 showed more expression in the gastric cancer II-IV stage group than in the stage I group (χ^2=19.54, p<0.01) (Table 5).

Relationship between α-Actin Expression and Invasion and Metastasis of Gastric Cancer

α-Actin expression was increased in the group with tumor invasion depth of T2-4 compared to the group with tumor invasion depth ≤T1 (χ^2=11.38, p<0.05). α-Actin had higher expression in gastric cancer with lymph node metastasis than without lymph node metastasis (χ^2=9.67, p<0.05). There was no significant difference in α-actin expression between gastric cancer without versus with distant metastasis (χ^2=5.57, p>0.05). α-actin expression showed no statistically significant difference between gastric cancer group stage I and stages II-IV (χ^2 = 6.46, p>0.05) (Table 6).

DISCUSSION

Relationship Between IGF-2, PCNA Expression and the Lauren Classification with Invasion and Metastasis of Gastric Cancer

IGF-2, a single chain of weak acid peptide, is encoded by the imprinted IGF-2 gene. It acts on the receptors (IGF-1R) through autocrine, paracrine or endocrine routes to regulate the proliferation and

Table 5. The relationship between MMP-7 expression and clinical pathologic parameters in gastric cancer

Variable	Group	Number	MMP-7 staining intensity			
			-	+	++	+++
Infiltration depth*	≤T1	20	8	9	3	0
	T2-4	30	2	6	12	10
Lymph node metastasis*	No	26	6	12	6	2
	Yes	24	4	3	9	8
Distant metastasis*	No	40	9	14	12	5
	Yes	10	1	1	3	5
TNM staging*	Stage I	27	8	13	5	1
	Stage II-IV	23	2	2	10	9

*There was a significant difference between MMP-7 expression and clinical pathologic parameters.

Table 6. The relationship between α-actin expression and clinical pathologic parameters in gastric cancer

Variable	Group	Number	α-Actin staining intensity			
			-	+	++	+++
Infiltration depth*	≤T1	20	3	9	6	2
	T2-4	30	0	5	18	7
Lymph node metastasis*	No	26	3	9	13	1
	Yes	24	0	5	11	8
Distant metastasis	No	40	3	13	19	5
	Yes	10	0	1	5	4
TNM staging	Stage I	27	3	10	11	3
	Stage I-II	23	0	4	13	6

*There was a significant difference between smooth muscle actin expression and clinical pathologic parameters.

differentiation of many tissues and cells through the PI3K/Akt signal transduction pathway (15). We found that IGF-2 was almost weakly expressed in normal gastric mucosa, and PCNA was negatively expressed in normal gastric mucosa. IGF-2 and PCNA were positively and strongly positively expressed in gastric carcinoma, suggesting that overexpression of IGF-2 in cancer cells can activate the signal transduction pathway in several ways, and continually transfer the signal of proliferation to cells, leading to continuous cell proliferation and malignant transformation.

Two studies about the relationship between IGF-2 expression and gastric cancer according to Lauren classification were reported. The immunohistochemical method was used to determine that the IGF-2 protein showed significantly higher expression in diffuse type gastric cancer than in intestinal type gastric cancer and that gastric cancer with overexpression of IGF-2 had late TNM staging (16). By way of in situ hybridization, it was shown that IGF-2 mRNA expression was higher in diffuse type gastric cancer than in intestinal type gastric cancer, and IGF-2 mRNA expression was relevant to the depth of tumor invasion, microvessel density, TNM stage, and lymph node and distant metastasis (17). In this study, adopting immunohistochemical methods, we found that there was no significant difference in IGF-2 expression intensity between diffuse type and intestinal type gastric cancer, but the expression patterns were significantly different: the majority of diffuse type gastric cancer had IGF-2 nuclear expression while the majority of intestinal type gastric cancer had IGF-2 expression in the cytoplasm. The change in IGF-2 expression patterns is related with the Lauren classification of gastric cancer, reflecting the molecular differences between the diffuse and intestinal type gastric cancer cells, which might be involved in the changes of IGF-2 gene and function.

We found that the gastric cancer with IGF-2 expression in the nucleus had deeper infiltration, more lymph node metastasis and later staging than that with IGF-2 expression in the cytoplasm. The molecular mechanisms by which the gastric cancer with IGF-2 expression in the nucleus is better able to invade and transfer than that with IGF-2 expression in plasma are unclear. However, it has been found that a variety of growth factors, such as epidermal growth factor and fibroblast

growth factor and their receptors, could be internalized by endocytosis and translocated to the cytoplasm, and they were reported to sometimes translocate from the cytoplasm to the nucleus in cancer cells to regulate transcription of specific target genes, induce a new signal pathway and relate to progression and poor prognosis of breast cancer and other tumors (18). At least two mechanisms have been found to explain this phenomenon: First, these factors can promote the proliferation and transformation of cancer cells after their nuclear translocation, and second, they can induce the resistance of cancer cells to radiotherapy and chemotherapy (18-20). IGF-2 nucleus translocation has not been reported currently, but its receptor IGF-1R has been shown to translocate to the nucleus. In the nucleus, it promoted transcription of specific target genes by direct binding to transcription factors or general coregulators (21). In vitro studies found that IGF could stimulate secretion of MMP-7 in the gastric cancer cell line, and when IGF-1R was blocked, MMP-7 secretion reduced to inhibit the invasion of cancer cells (22). This study showed that the vast majority of diffuse type gastric cancer showed IGF-2 expression in the nucleus, and PCNA and MMP-7 expressions were increased compared with intestinal type gastric cancer. Whether IGF-2 expression in the cancer cell nucleus can enhance invasion and metastasis by promoting cancer cell proliferation and MMP-7 secretion deserves further study.

Relationship Between MMP-7 Expression and the Lauren Classification with Invasion and Metastasis of Gastric Cancer

Matrix metalloproteinases (MMPs) are a group of zinc-dependent proteolytic enzymes. MMP-7 is a highly active MMP family member, and can activate other family members, such as MMP-2 and MMP-9, and play a central role in the degradation of the extracellular matrix (5). It also inhibits apoptosis of cancer cells, reduces cell adhesion and induces angiogenesis, making it easier for the cancer cells to invade small blood vessels and lymphatic tube and metastasize (23-25). Our study also confirmed that MMP-7 was not expressed in normal gastric mucosa, and MMP-7 expression significantly increased in gastric cancer cells. MMP-7 overexpression was related to the depth of tumor invasion, lymph node metastasis, distant metastasis, and staging.

We found that the MMP-7 expression level was higher in diffuse type than in intestinal type gastric cancer. Compared with intestinal type gastric cancer, more changes in gene expression were found in diffuse type and involved epithelial cells and extracellular matrix interactions, such as genes of extracellular matrix components (including MMP), smooth muscle and cell adhesion molecules. Consistent with results of gene expression studies, immunohistochemistry has shown that the extracellular matrix and basement membrane component had lower expression in diffuse type gastric cancer than intestinal type (26). E-cadherin is one of the most important intercellular adhesion molecules in the epithelial tissue. The genetic changes in diffuse gastric cancer can bring about low expression or dysfunction of encoded E-cadherin in order to reduce cell adhesion, making it easier for cancer cells to spread to the depth in the form of dispersedness, thus leading to a poor prognosis. Research on the gastric cancer cell line found that secretion of MMP-7 in cancer cells increased and reduced the expression of E-cadherin in cancer cells to promote cancer cell invasion and metastasis through mediating the shedding of E-cadherin extracellular functional areas (27). Diffuse type gastric cancer has higher MMP-7 expression than intestinal type, which may be related to E-cadherin dysfunction in diffuse type gastric cancer.

Relationship Between α-Actin Expression of Myofibroblasts and the Lauren Classification with Invasion and Metastasis of Gastric Cancer

We found a small amount of scattered myofibroblasts in normal gastric mucosa, and α-actin expression was increased significantly in gastric cancer and irregularly distributed among the cancer nests. Nakayama et al. (28) found that myofibroblast positivity in intestinal type gastric cancer was higher than in diffuse type. This study showed α-actin expression had no relation with Lauren classification, suggesting that interstitial myofibroblasts showed no significant differences between intestinal type and diffuse type gastric cancer. It was also noted that there was no significant interstitial myofibroblast proliferation in a small number of diffuse, poorly differentiated gastric cancer cases, which may reflect individual differences in the pathogenesis of gastric cancer. It has been shown that there were large differences in the interstitial myofibroblast number among different cases of breast and bladder cancer[29,30]. Some studies have found that α-actin

expression is related to the depth of invasion or lymph node metastasis of the gastric cancer, suggesting that gastric interstitial myofibroblasts were involved in gastric cancer invasion and metastasis (29,31). The research on the molecular mechanisms of myofibroblasts promoting tumor progression have revealed that tumor-associated myofibroblasts, by participating in synthesis, deposition and remodeling of the extracellular matrix, producing some proteases, such as fibroblast activation protein, matrix metalloproteinases and urokinase plasminogen activator, promoting tumor angiogenesis, secreting soluble paracrine signals to stimulate proliferation of the tumor cell, and inducing tumor immune escape, thereby promoted tumor development (32-38).

In summary, our study determined that there are significant differences in the expression patterns of IGF-2 and expression intensity of PCNA and MMP-7 between diffuse type and intestinal type gastric cancer cells, but no significant differences in the expression intensity and expression patterns of myofibroblasts, suggesting that the clinical pathologic disparity of intestinal type and diffuse type gastric cancer may be due mainly to changes in genetic structure and phenotypic characterization of epithelial cells, and that interstitial myofibroblasts co-promote invasion and metastasis of gastric cancer in coordination with cancer cells.

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