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Original Article

Expression of Snail and Twist Compared with Clinical and Pathological Parameters in Patients with Gastric Cancer

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Abstract

Introduction: Epithelial-mesenchymal transition (EMT) is a process of change in the cellular phenotype from epithelial to mesenchymal morphology. The changes at the cellular level can explain the great heterogeneity and plasticity in the different histological subtypes of gastric carcinomas, which causes difficulties in therapy. In it, epithelial cells reduce intercellular adhesion, which is crucial in the process of invasion and metastasis of gastric carcinomas. Inhibition of cell adhesion molecules such as E-cadherin is known to be influenced by a number of transcription factors, such as Snail and Twist.

Materials and methods: Our study aims to examine immunohistochemically the expression of the transcription factors Snail and Twist in 69 patients with gastric cancer and to look for a link between their expression and clinical and pathological characteristics.

Results: Positive expression of Snail and Twist was observed in all cases studied by us. We observed heterogeneity and different intensity of immunohistochemical expression. There is a correlation between the immunohistochemical expression and the degree of differentiation of tumor cells and the tumor stage. The cells of poorly differentiated adenocarcinomas show diffuse and strong nuclear staining. No correlation was found between the expression of the two markers, age, and sex of the patients.

Conclusions: Evaluating the expression of the two markers studied may help to assess tumor progression and prognosis. They can be used for more accurate and effective diagnosis in precancerous lesions and in early gastric cancer because they are not expressed in the normal gastric mucosa.

Keywords

EMT, gastric cancer, progression, Snail, Twist

INTRODUCTION

Gastric cancer is one of the most common and deadly cancers globally; it is the fifth most common cancer in the world.[1] Most gastric carcinomas are adenocarcinomas with varying degrees of cell differentiation. Laurén's classification divides gastric adenocarcinomas into two main types: the one that presents a structure similar to colon cancer is defined as intestinal; the other that is specific for stomach cancer and more common in younger people is defined as diffuse.[2]

The intestinal type adenocarcinoma is more common than the diffuse type. The intestinal type is characterized by the presence of glandular structures with mitotically active cylindrical cells. These tumors often occur in older populations and are twice as common in men as in women.[3]

Intestinal adenocarcinomas are usually located in the antrum of the stomach and tend to spread hematogenously. Diffuse adenocarcinomas are characterized by small ring-shaped cells (signet-ring cell) that are uniform in shape and nuclear in size and exhibit lower mitotic activity. Gastric tumors of this type are more common in the gastric corpus and are recognized by the lack of glandular formation and cell adhesion.[4]

Due to its complex mechanisms of initiation and progression of gastric cancer, its early detection and effective treatment is hardly possible.[5] Gastric cancers show marked heterogeneity in terms of tumor growth, histogenesis, and molecular pathogenesis.[6] In the process of transcriptional regulation, several key transcription factors (including Snail, Twist, and ZEB) play roles that repress E-cadherin expression and are expressed selectively in gastric cancer.[7] The transcription factors Twist and Snail are immediate activators of the epithelial-mesenchymal transition in tumor cells. The Snail family members of protein play a central role in the regulation of EMT during tumor progression by repressing E-cadherin transcription.[8] Twist-related protein was recently reported as an important regulator of carcinogenesis and tumor metastasis in different solid tumors, including gastric cancer. Twist and Snail expression is associated with invasive carcinoma proliferation and is a poor prognostic factor.[9] Snail induces EMT by directly suppressing E-cadherin expression, leading to tumor cell differentiation and maintenance of the invasive phenotype.[10] Twist expression in gastric cancer is significantly associated with vascular invasion, positive lymph nodes, and distant metastases.[11] High Twist and Snail expression is associated with malignant features of gastric cancer. Evaluating the expression of the two markers under investigation may help in assessing the tumor progression providing a more accurate and effective diagnosis and prognosis.

AIM

The present study was designed to evaluate the Twist and Snail immunohistochemical expression in a series of patients with intestinal or diffuse gastric cancer and compare it with some clinical and pathological indicators.

MATERIALS AND METHODS

The study included a retrospective and prospective investigation of 69 cases of patients with gastric cancer over a period of 3 years (2018-2020), 49 men and 20 women in the age range of 35-87 years. Of these, 56 had intestinal gastric cancer and 13 had the diffuse type of gastric cancer.

All patients were operated on and diagnosed in the Clinic of Surgery and the Clinic of Gastroenterology of Kaspela University Hospital and Pulmed University Hospital of Plovdiv. The biopsy materials were reviewed by two independent pathologists to diagnose and stage the tumor.

The histological and immunohistochemical investigation of the biopsy materials was performed in the laboratory of the Department of General and Clinical Pathology of the Medical University in Plovdiv.

Immunohistochemical analysis

The tissue was treated with standard histopathological technique according to standard protocols, and semi-quantitative analysis of the results was performed. Five-μm thick paraffin sections were dewaxed and rehydrated through descending alcohols. Hematoxylin-eosin staining was performed according to standard methods.

The immunohistochemical study was performed according to standard protocols. Antibodies produced by Quartett, Germany and Biorbyt, Great Britain were used:

- rabbit polyclonal antibody against human Twist. We reported nuclear staining.
- rabbit polyclonal antibody against human Snail. Cytoplasmic and nuclear staining were detected.

DAB was used as the chromogen. An automated coloring platform was used.

Expression estimation system

A semi-quantitative method was used to assess the immunohistochemical expression: >5% = 0; 5-25% = 1; 25-50% = 2; >75% = 3.

Intensity of immunohistochemical expression: 0 = missing; 1 (+) = weak; 2 (+) = moderate; 3 (+) = strong.

The immunohistochemical expression is the sum of the percentage of positive cells and the intensity. At least 10 ×400 magnification fields from each preparation were examined.

All cases were reviewed by two pathologists for confirmation of diagnosis, staging, and grading.

Statistical analysis

Comparison of Twist and Snail expression and clinicopathological parameters was investigated by chi-square test or Fisher's exact test, when appropriate. A p-value lower than 0.05 was considered statistically significant. SPSS version for Windows 13.0 was used to analyze the survey data.

RESULTS

Positive expression of Snail and Twist was observed in all cases studied **(Fig. 1)**. There is a correlation between the immunohistochemical expression and the degree of differentiation of tumor cells and the tumor stage. The cells of moderately and poorly differentiated adenocarcinomas show diffuse and strong nuclear staining. In these cases, the expression of E-cadherin is weak and heterogeneous. Negative expression of both markers was observed in the preserved gastric mucosa adjacent to the tumor tissue.

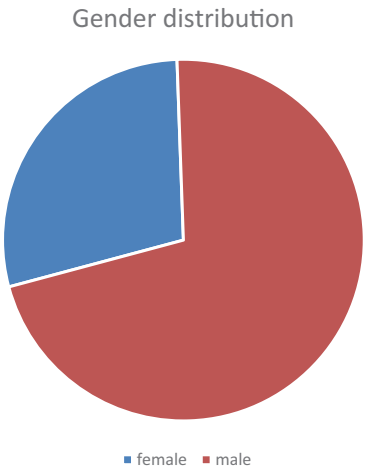

Figure 1. Distribution of patients by sex.

Immunohistochemically, the expression of Snail and Twist was statistically analyzed to determine their relationship to the clinical and pathological features of the gastric cancer patients we studied **(Figs 2, 3)**. The results showed that overexpression of both markers strongly correlated with tumor cell differentiation ($p<0.05$), with the TNM stage of the tumor ($p<0.001$), with the presence of lymph node metastases, and with distant metastases in the liver.

Positive expression did not correlate with age, sex, tumor location, and tumor size. In connection with the two main types of gastric cancer, intestinal and diffuse type according to Laurén's classification, differences in the degree of expression of Snail and Twist were associated with low differentiation groups of carcinomas in both types ($p<0.05$) **(Tables 1, 2)**.

Men predominate over women in a ratio of 2.5 to 1.

The largest number of studied cases with respect to pT-stage was in pT3-stage in 36 cases, pT4-21, pT2-12. Regarding pN- and pM-stages, we studied 7 patients with lymph node metastases and 2 patients with liver metastases. In cases of gastric cancer metastases, the expression level of Twist and Snail was identical to that in the primary tumor. Correlations among the expressions of Twist and Snail were immunohistochemically examined in gastric cancers and adjacent normal tissues. In normal gastric tissues, positive immunohistochemical expression was not reported for both immunohistochemical markers in the biopsy specimens examined.

Table 1. Distribution of the study groups by Twist expression in different type of gastric cancer (%)

N	Diffuse type	Intestinal type
Strong TWIST expression	2 (15%)	6 (10%)
Moderate TWIST expression	9 (70%)	27 (48%)
Weak TWIST expression	2 (15%)	23 (42%)
Negative TWIST expression	0	0
Total	13 (100%)	56 (100%)

Table 2. Distribution of the study groups by Snail expression in different type of gastric cancer (%)

N	Diffuse type	Intestinal type
Strong SNAIL expression	2 (15%)	3 (5%)
Moderate SNAIL expression	7 (55%)	27 (49%)
Weak SNAIL expression	4 (30%)	26 (46%)
Negative SNAIL expression	0	0
Total	13 (100%)	56 (100%)

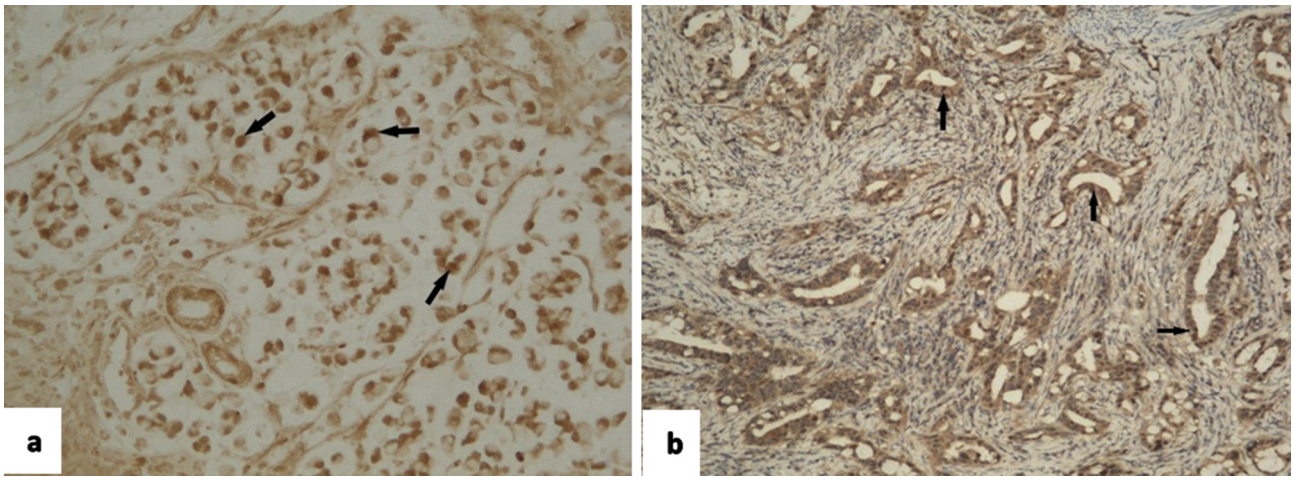

Figure 2. Expression of Snail in diffuse and intestinal type carcinoma of the stomach. (**a**) immunohistochemical expression of Snail in signet-ring cell gastric cancer (high-intensity immunoreactivity of more than 10% of the tumor cells, 3+, localizing in the cell nucleus and cytoplasm), ×100; (**b**) immunohistochemical expression of Snail in intestinal-type gastric cancer (strong positive expression, 3+ in moderately differentiated adenocarcinoma cancerous tissue, localizing in the cell nucleus and cytoplasm), ×100.

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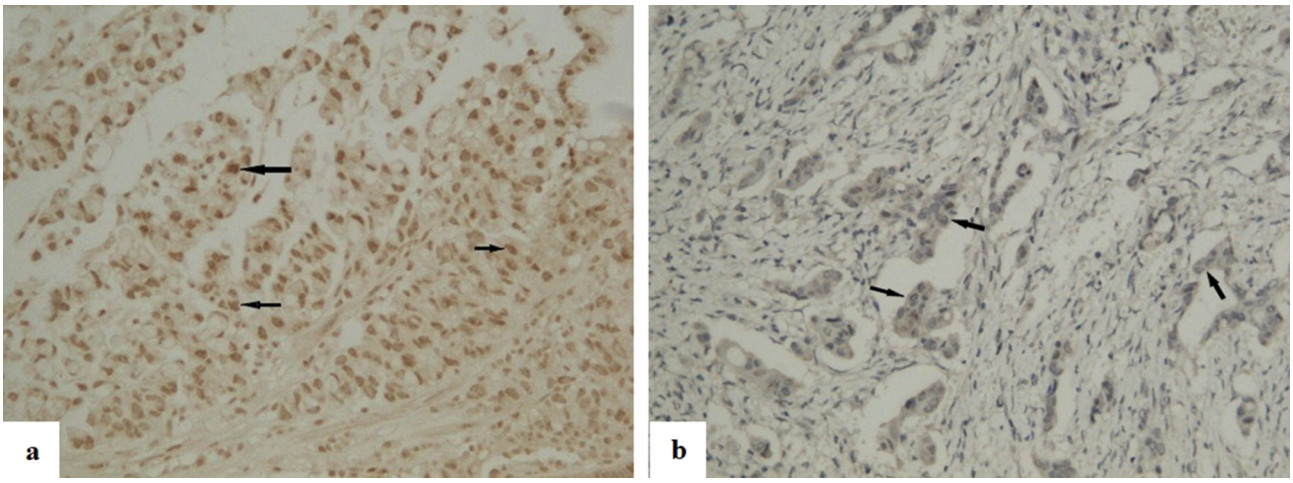

Figure 3. Twist expression in diffuse and intestinal type of gastric cancer. (**a**) immunohistochemical expression of Twist in diffuse type gastric carcinoma (moderate-intensity immunoreactivity of more than 10% of the tumor cells, 2+, localizing in the cell nucleus), ×100; (**b**) immunohistochemical expression of Twist in intestinal type gastric carcinoma (moderate-intensity immunoreactivity of more than 10% of the tumor cells, 2+, localizing in the cell nucleus), ×100.

The expression of both studied markers remained high in both intestinal and diffuse carcinomas of the stomach. Decreased cell differentiation increased the Twist and Snail expression. All cases with poor immunohistochemical expression were highly differentiated. Twist expression was higher in low-grade intestinal carcinomas than in Snail's, and vice versa in diffuse carcinomas.

DISCUSSION

The EMT process is always accompanied by a loss of cell-cell adhesion mediated by E-cadherin, which plays a critical role in maintaining cellular polarity and epithelial integrity.[12] E-cadherin transcription in vitro and in vivo can be suppressed by the zinc-finger transcription factor Snail, which has been reported to bind to the sequence in the E-cadherin promoter.[8] Snail-expressing cells become invasive, supporting its role in tumor progression.[13] Snail expression has been extensively studied in various human cancer tissues and cell lines.[14-16] Snail expression is significantly more pronounced in cancerous tissues than in normal stomach tissue.[17,18]

Among the transcription factors that are overexpressed in metastatic gastric cancer, Twist was the first to be selected for the study. Ryu et al.[19] used real-time quantitative PCR and immunohistochemistry to analyze the relationship between the Twist expression and tumors in 436 cases of gastric cancer, and their results showed that Twist expression levels in tumor tissues and metastatic lymph nodes were overregulated in comparison to normal gastric mucosa. Twist expression in gastric cancer has been shown to be significantly associated with positive lymph nodes and distant metastases.[20] The results of our study are similar.

We did not observe negative expression of Twist and Snail in tumor cells in diffuse and intestinal carcinomas, but in four of the cases of low-grade carcinomas, we reported heterogeneous expression of variable intensity, which may be due to intratumoral hypoxia. In addition, the overexpression of Twist, at the edges of the tumor nidus and tissues adjacent to tumors reported by other authors, shows inconsistencies in the literature on how this expression changes as the disease progresses. Our study did not confirm similar results. The small number of cases studied in our sample may be the reason for this, which requires further confirmation.

Although a number of studies report Twist and Snail expression only in the cytoplasm and nuclei of tumor cells, the exact explanation for the significance of this biological finding in the progression from normal mucosa to carcinoma remains unclear.[21-23]

In our study, a positive expression of Twist and Snail was observed predominantly in the cytoplasm and/or nuclei of tumor cells in gastric carcinomas, with an increase in intensity in tumors with higher stages of pTNM.

The transcription factors Twist and Snail are immediate activators of the epithelial-mesenchymal transition in tumor cells. Twist and Snail expression is associated with invasive carcinoma proliferation and is a poor prognostic factor. Snail induces EMT by directly suppressing E-cadherin expression, leading to tumor cell differentiation and maintenance of the invasive phenotype.[24] Twist is also associated with the clinical course of gastric cancer, as it is significantly more expressed in tumors at higher TNM stages. Similar results have been observed by other authors.[25]

Snail induces EMT by directly suppressing E-cadherin expression.[26] Inhibition of Snail expression can lead to high expression of E-cadherin, which has a significant effect on the metastatic properties of cancer cells. Snail is significantly more pronounced in cancerous tissues than in normal tissue.

Positive expression of Snail and Twist was observed in all cases studied by us. There is a correlation between immunohistochemical expression and the degree of differentiation of tumor cells and the tumor stage. The cells of poorly differentiated adenocarcinomas show diffuse and strong nuclear staining. Negative expression of both markers was observed in the preserved gastric mucosa adjacent to the tumor tissue. No correlation was found between the expression of the two markers, age, and sex of the patients. In the era of personalized medicine, the expression of molecular markers is essential for the diagnosis, treatment, and prognosis in patients with gastric cancer.[27]

CONCLUSIONS

The results of our study confirm the role of transcription factors in the progression of blood cancer. In our study, in all cases of gastric cancer, we observed positive expression of Snail and Twist in both tumor cell nuclei and cytoplasms. Data from the present study show that Snail and Twist overexpression correlates with clinical progression in patients with gastric cancer, especially with respect to cell differentiation, lymph node metastases, distant metastases, and TNM stage of the tumor.

Many proteins can be used as early diagnostic markers of gastric cancer, while the regulation of EMT can provide potential targets for therapy. Snail and Twist can be used as independent diagnostic markers in borderline proliferative lesions of the stomach because they are expressed only in tumor tissue.

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Экспрессия Snail и Twist в сравнении с клинико-патологическими параметрами у больных раком желудка

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Резюме

Введение: Эпителиально-мезенхимальный переход (ЭМП) представляет собой процесс изменения клеточного фенотипа с эпителиальной на мезенхимальную морфологию. Изменения на клеточном уровне могут объяснить большую гетерогенность и пластичность различных гистологических подтипов рака желудка, что вызывает трудности в терапии. В нём эпителиальные клетки снижают межклеточную адгезию, что имеет решающее значение в процессе инвазии и метастазирования карциномы желудка. Известно, что на ингибирование молекул клеточной адгезии, таких как Е-кадгерин, влияет ряд факторов транскрипции, таких как Snail и Twist.

Материалы и методы: Целью нашего исследования было иммуногистохимическое изучение экспрессии факторов транскрипции Snail и Twist у 69 больных раком желудка и поиск связи между их экспрессией и клинико-патологическими характеристиками.

Результаты: Положительная экспрессия Snail и Twist наблюдалась во всех исследованных нами случаях. Мы наблюдали гетерогенность и различную интенсивность иммуногистохимической экспрессии. Существует корреляция между иммуногистохимической экспрессией и степенью дифференцировки опухолевых клеток и стадией опухоли. Клетки низкодифференцированных аденокарцином демонстрируют диффузное и сильное ядерное окрашивание. Корреляции между экспрессией двух маркеров, возрастом и полом пациентов обнаружено не было.

Заключение: Оценка экспрессии двух изученных маркеров может помочь оценить прогрессирование опухоли и прогноз. Их можно использовать для более точной и эффективной диагностики предраковых состояний и раннего рака желудка, поскольку они не экспрессируются в нормальной слизистой оболочке желудка.

Ключевые слова

ЭМП, рак желудка, прогрессирование, Snail, Twist