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Promotive effect of Talin-1 protein on gastric cancer progression through PTK2-PXN-VCL-E-Cadherin-CAPN2-MAPK1 signaling axis

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Abstract

Objective: Our research group was aim to explore the molecular mechanism of Talin-1 protein affecting gastric cancer progression through PTK2-PXN-VCL-E-Cadherin-CAPN2-MAPK1 signal axis.

Methods: 12 cases of patients with gastric cancer in this hospital from 2018 to 2019 were collected. Immunohistochemistry assay and Western blotting were used to detect the expression of Talin-1, PXN, E-Cadherin, CAPN2, MAPK1 protein in gastric cancer tissue. Cell migration and invasion were measured by Transwell.

Results: The results showed that the expression levels of protein Talin-1, PXN and MAPK1 in gastric cancer tissues were significantly higher than that in normal tissue. The number of cell adhesion in the model group was significantly lower than that in the normal group. However, the cell adhesion number in ov-TLN1 was the highest. Transwell results showed that TLN1 could accelerate the migration and invasion abilities of gastric cancer MKN-45 cells. Moreover, Western blotting showed that protein Talin-1, PXN, E-Cadherin, CAPN2, MAPK1 in model group all increased compared with normal group.

Conclusion: It indicated that talin-1 protein influenced the development of gastric cancer through PTK2-PXN-VCL-E-Cadherin-CAPN2-MAPK1 signal axis.

KEYWORDS
gastric cancer, mechanism, MKN-45 cells, signaling axis, talin-1

1 | INTRODUCTION

Gastric cancer is one of the most common gastrointestinal malignancies. In the world, the incidence of gastric cancer ranks the fourth in malignant tumors and the death rate ranks the second in malignant tumors.[1] China is a country with a high incidence of gastric cancer, mostly occurring over the age of 40 years old, about two-third of them are aged from 41 to 60 and the ratio of male to female is about 3.6 to 1. Every year, there are about 400 000 new cases of gastric cancer, and at the same time, about 300 000 people die of gastric cancer, with the mortality rate ranking among the top of all kinds of tumors.[2,3] Therefore, gastric cancer has become a serious threat

to people's life and health and brought a heavy economic burden for patients and the society. At present, the therapy of gastric cancer mainly includes surgery and traditional chemoradiotherapy. However, due to the high recurrence and metastasis rate after gastric cancer treatment, molecular targeted drugs have not been widely used in gastric cancer, so now the clinical treatment effect is not ideal. Research on the mechanism of gastric cancer recurrence and the scientific problem of reducing recurrence have been paid more attention. An in-depth understanding of these related studies not only provides a molecular explanation for the recurrence of gastric cancer, but also provides a theoretical basis for prevention the recurrence of gastric cancer.

At present, researches have shown that adhesion and invasion of malignant cells are related to various signaling pathways in vivo, including integrin receptor signaling pathway, growth factor receptor signaling pathway, and mitochondrial autophagy related pathway, among which integrin-activated adhesion kinase (FAK) mediating signaling pathway is an important pathway for tumor invasion and metastasis.[4] Talin-1 protein is a cytoskeleton protein that can bind to integrins and is involved in integrin-mediated cell adhesion and tumor genesis and development. PTK2 protein is a kind of adhesion protein kinase, which mainly regulates cell adhesion, migration, and exocytosis.[5-7] PXN protein is involved in regulating the adhesion between membrane proteins and extracellular matrix. However, VCL protein regulates intercellular adhesion, cell-matrix adhesion, and expression of E-cadherin as an adhesion protein.[8] E-cadherin protein is an important tumor metastasis inhibited gene involving in maintaining the normal epithelium integrity and polarity. When the cell integrity is destroyed and the polarity is disturbed, the tumor cells become more active and their activity range is enlarged, which makes it easier for tumor cells to break away from the primary site to invade and metastasize.[9] CAPN2 protein is involved in cytoskeleton remodeling and signal transduction, while MAPK1 is involved in cell proliferation and differentiation.[10] Based on the above theory of the role of protein factors in malignant tumors, our study aim to explore the molecular mechanism of talin-1 protein affecting gastric cancer progression through PTK2-PXN-VCL-E-cadherin-CAPN2-MAPK1 signal axis.

2 | EXPERIMENTAL DESIGN AND MATERIALS

2.1 | Materials

Gastric cancer MKN-45 cell and normal gastric mucosa GES-1 cells (ATCC Cell Bank); 5-acetylene-2′-deoxyuridine (EdU) detection kit (Guangzhou Ruibo Biological Technology Co. LTD); fetal bovine serum (FBS); DMEM culture medium (Gibco); CCK-8 cell count kit (Wuhan Mershark Biotechnology Co. LTD); BX3 fluorescence microscope (Olympus); ABI 7500 fluorescence quantitative PCR instrument (Applied Biosystems); iMark multimode reader (Bio-Rad).

2.2 | Methods

2.2.1 | Patients

Twelve cases of patients with gastric cancer in this hospital from 2018 to 2019 were collected. Gastric cancer tumor tissue from patients undergoing tumor resection and paired para-cancer control tissue were collected (2.5 cm from the edge of tumor tissue). This research was approved by the Hospital ethics Committee (No 20180212348).[11]

2.2.2 | Immunohistochemistry assay

Twelve cases of gastric cancer and 12 cases of normal gastric tissue samples were collected, respectively. All patients were performed gastrectomy for gastric cancer. During the operation, gastric cancer tissues more than 1 cm from the tumor edge and para-cancer tissues more than 5 cm from the tumor edge were retained. The remaining part of the specimen is stored in liquid nitrogen at −80°C. Immunohistochemistry assay was used to measure the expression and distribution of talin-1, PTK2, and E-cadherin in gastric cancer tissue samples. After conventional dewaxation, sections were repaired by microwave antigen of citric acid buffer solution, incubated with CK (AE1/AE3) antibody at room temperature for 1 hour, incubated with two antibodies at room temperature for 30 minutes, colored by DAB, re-stained with hematoxylin, dehydrated with anhydrous alcohol, and sealed with neutral gum for observation.[12]

2.2.3 | Expression of protein talin-1, PXN, E-cadherin, CAPN2, MAPK1 in gastric cancer tissue samples

Western blotting was used to detect the expression of talin-1, PXN, E-cadherin, CAPN2, MAPK1 protein in gastric cancer tissue, and gastric cancer adjacent tissue (normal) groups. The protein was extracted with RIPA lysate. The proteins were separated by SDS-polyacrylamide gel electrophoresis. After the protein was transferred and sealed, the primary antibody (talin-1, PXN, E-cadherin, CAPN2, MAPK1 and β-actin) was incubated at 1:1000 dilution for 2 hour at room temperature. After TBST washing, HRP-labeled secondary antibodies were incubated at room temperature for 1 hour. talin-1, PXN, E-cadherin, CAPN2, and MAPK1 protein bands in each group were scanned with ImageJ software and quantified with β-actin gray value.[13]

2.2.4 | Adhesion detection

Cells from the NC group, model group, ov-TLN1, si-TLN1, and blank vector groups were collected, respectively, and inoculated into endothelial cells with a concentration of 5×10^4/well. The cells were incubated at 37°C in incubator with 5% CO_2 for 30 minutes. The culture medium was discarded. Unadherent gastric cancer cells

were washed twice with DMEM culture medium of 10% FBS. 100 μL 0.25% Rose Bengal staining solution was added to each well and incubated for 5 minutes at room temperature and then washed twice. 200 μL 95% ethanol: PBS (1:1) solution was added to each well and incubated at room temperature for 30 minutes. The condition of the cells in the culture plate was observed and photographed under microscope, and the absorbance value of cells at 570 nm was determined using enzyme marker.[14]

2.2.5 | Cell migration and invasion were measured by Transwell

The cells of NC group, model, ov-TLN1, si-TLN1, blank vector group were cultured. Cell suspension with concentration of 5×10^5/mL was prepared by serum-free medium for each group. 100 μL single-cell suspension was added to the upper chamber of Transwell, and 600 μL DMEM containing 10% fetal bovine serum was added to the lower chamber for incubating 48 hours. The upper compartment was removed and erased; then, the cells were stained with 0.25% crystal violet for 10 minutes. Five cell images were taken and counted under the microscope using the random field method.[15]

2.2.6 | Western blotting

Cells of NC group, model, ov-TLN1, si-TLN1, and Blank vector were collected, respectively. Western blotting was used to detect the expression of talin-1, PXN, E-cadherin, CAPN2, MAPK1 protein in different groups. The protein was extracted with RIPA lysate. The proteins were separated by SDS-polyacrylamide gel electrophoresis. After the protein was transferred and sealed, the primary antibody (talin-1, PXN, E-cadherin, CAPN2, MAPK1 and β-actin) was incubated at 1:1000 dilution for 2 hours at room temperature. After TBST washing, HRP-labeled secondary antibodies were incubated at room temperature for 1 hour. talin-1, PXN, E-cadherin, CAPN2, and MAPK1 protein bands in each group were scanned with ImageJ software and quantified with β-actin gray value.[16]

3 | RESULTS

3.1 | Immunohistochemistry assay

Immunohistochemical positive infection showed a brown color and was located in the cell membrane and cytoplasm, which indicated that MKN-45 cells were infected. The expressions and distribution of
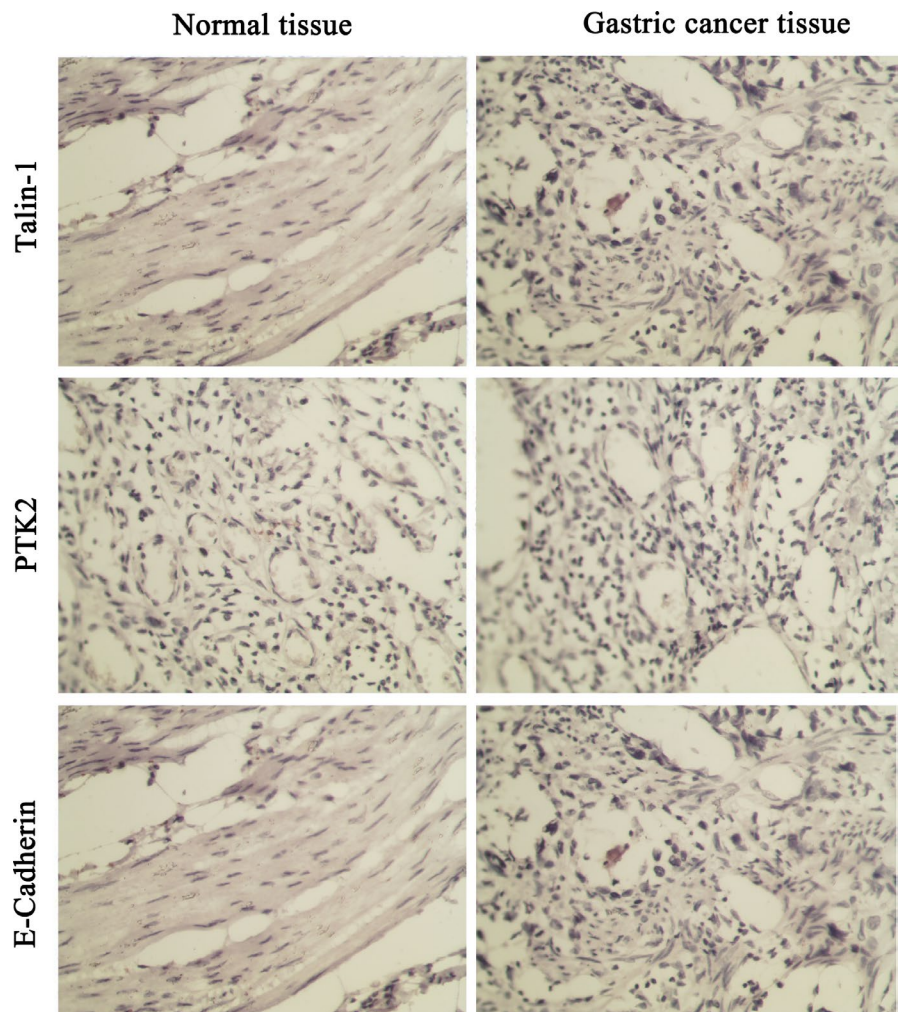
FIGURE 1 Expression and distribution of talin-1, FAK1 (PTK2), and E-cadherin in relevant tissue samples (Magnification 200×)

proteins talin-1, PTK2, and E-cadherin in relevant tissue samples are shown in Figure 1. Talin-1, PTK2, and E-cadherin are the main components in cells. Immunohistochemical results showed that the expression level of talin-1 and PTK2 in gastric cancer tissues was higher than that in the normal tissue group, while the expression level of E-cadherin in gastric cancer tissues was lower than that in the normal tissue group.

3.2 | Expression of protein talin-1, PXN, E-cadherin, CAPN2, MAPK1 in gastric cancer tissue samples

The expression levels of protein talin-1, PXN, and MAPK1 in gastric cancer tissues were significantly higher than that in corresponding adjacent tissues (normal tissue), showing significant statistical differences ($P < .01$). However, the expression level of E-cadherin and CAPN2 in gastric cancer tissues was significantly lower than that in the normal tissues ($P < .01$), as shown in Figure 2.

3.3 | Adhesion detection

The number of cell adhesion in the model group was significantly lower than that in the normal group. However, the cell adhesion number in ov-TLN1 was the highest. However, after the gastric cancer MKN-45 cells adding si-TLN1, the adhesion ability of gastric cancer cells decreased significantly. As shown in Figure 3, the trend of cell adhesion ability in the experimental group was greater of ov-TLN1 than that of model group, than that of the Blank vector group, than that of the si-TLN1 group.

3.4 | Cell migration and invasion were measured by Transwell

The results of Transwell indicated that the number of migration cells was significantly increased in model group compared with normal group, among which the number of migration cells was ov-TLN1 group > Model group > Blank vector group > si-TLN1 group > NC group. As shown in Figure 4, the results showed that TLN1 can accelerate the migration and invasion abilities of gastric cancer MKN-45 cells.

3.5 | Western blotting

The expression of proteins talin-1, PXN, E-cadherin, CAPN2, MAPK1 was detected by Western blot. As shown in Figure 5, protein talin-1, PXN, E-cadherin, CAPN2, MAPK1 in gastric cancer (model) group all increased compared with normal (NC) group $**P < .01$, $***P < .001$. The trend of the expressed level of proteins talin-1, PXN was ov-TLN1 group > model group > Blank vector group > si-TLN1 group, $\#P < .05$, $\#\#P < .01$, compared with model group. However, the trend of the expressed level of proteins E-cadherin, CAPN2 was si-TLN1 group > Blank vector group > model group > ov-TLN1 group.

4 | DISCUSSION

Talin is a kind of macromolecular cytoskeleton protein, which is an important component in the adhesion center of cells, and can bind to many kinds of cell adhesion molecules. Talin activates integrin and plays a key regulatory role in cell adhesion and migration as a bridge between cytoskeleton and transmembrane receptor integrin. Talin is the first proven integrin-binding cytoskeleton protein, which is involved in integrin-mediated cell adhesion, metastasis, and diffusion, and promotes the development of tumors.[17] Talin has two ankle protein genes in vertebrates, which are talin-1 and talin-2, respectively. Studies have shown that talin-2 is the starting gene, and its function are still unknown. Talin-1 is mainly expressed in human kidneys, liver, spleen, stomach, lungs, and vascular smooth muscle.[18] Recent studies have shown that talin-1 is the most important FAP component. It connects integrin and actin and, as the main protein of FAP, connects various adhesion
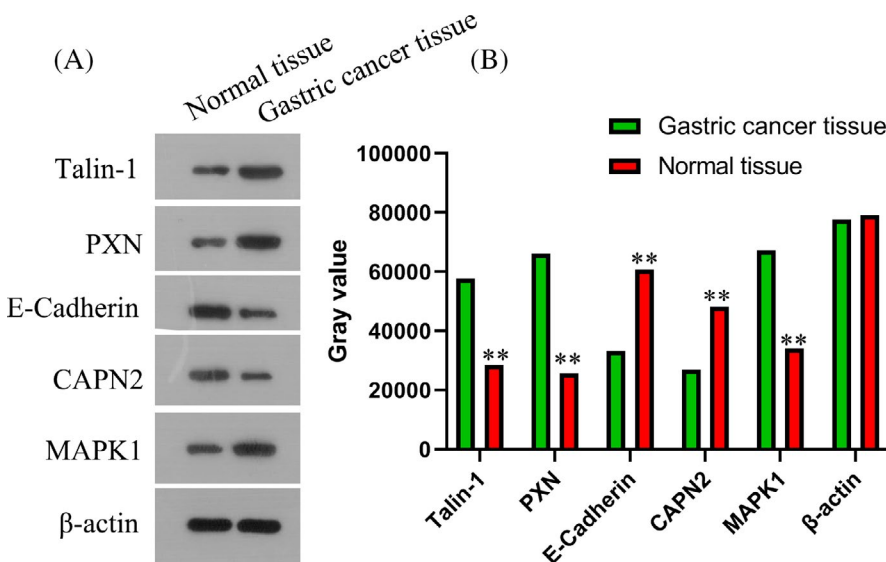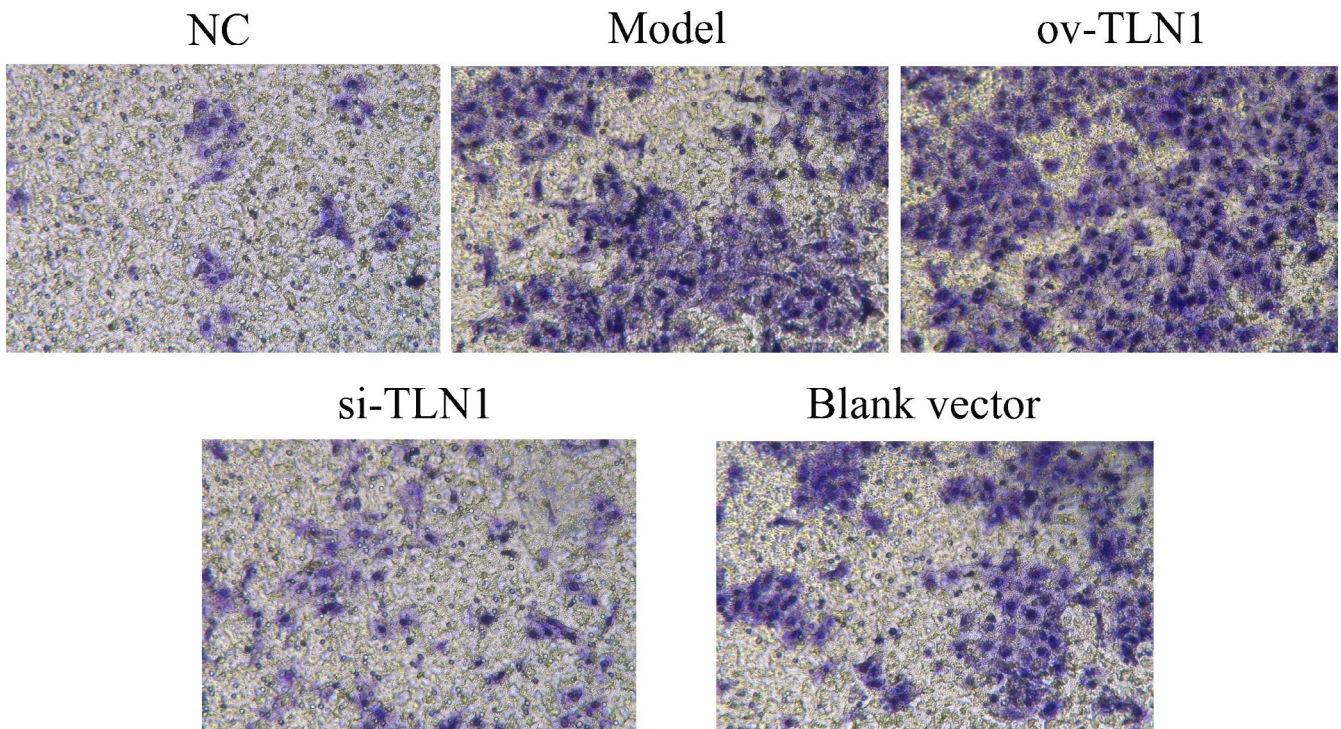

FIGURE 2 The expression levels of talin-1, PXN, E-cadherin, CAPN2, MAPK1 protein of gastric cancer tissue and corresponding adjacent non-cancerous tissues (normal tissues). $**P < .01$ was considered significantly difference compared with normal tissues
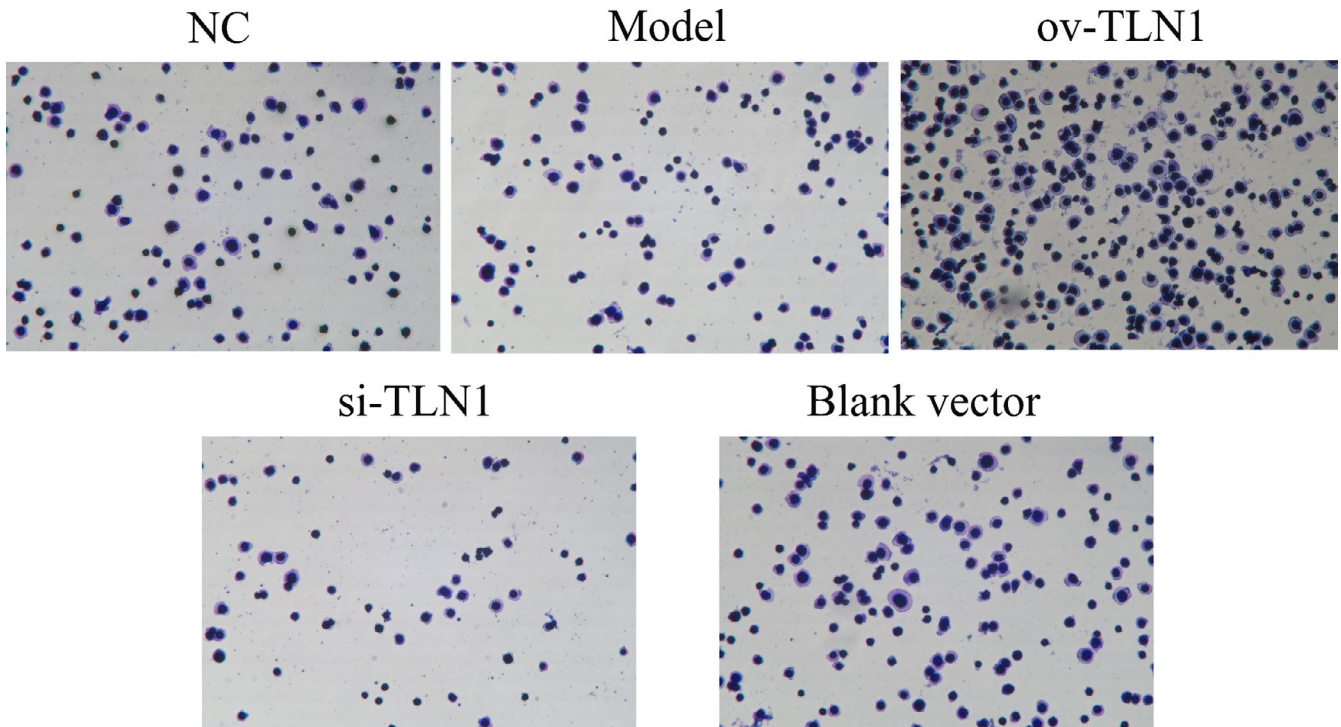
FIGURE 3 Adhesion assay of gastric cancer MKN-45 cells in each group (Magnification × 100)

FIGURE 4 Cell invasion of gastric cancer MKN-45 cells was measured by Transwell (Magnification × 100)

molecules and plays an important role in the process of cell adhesion and migration. The results of this experiment showed that talin-1 expression was low in normal gastric tissue, but high in gastric cancer. Studies have shown that talin-1 plays an important regulatory role in tumor metastasis and invasion.

During integrin-mediated signal transduction, integrins bind to ECM, cytoskeleton proteins, signal transduction molecules, and other proteins, thus mediating two-way information transmission between inside and outside the cell.[19] Talin has two binding sites to integrin, and talin's homologous dimer has four binding sites to integrin, thus
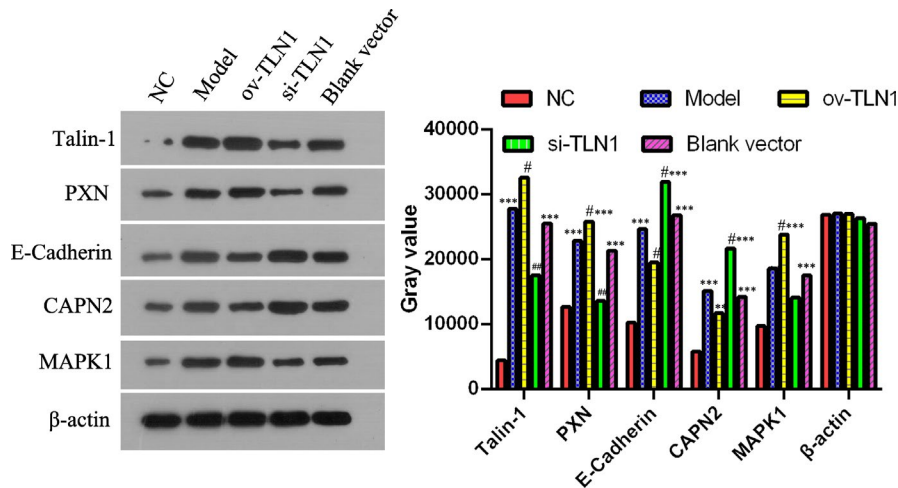
FIGURE 5 The expression levels of talin-1, PXN, E-cadherin, CAPN2, MAPK1 protein of gastric cancer tissue and corresponding adjacent non-cancerous tissues (normal tissues). $**P < .01$, $***P < .001$ was considered significantly difference compared with normal group. $^{\#}P < .05$, $^{\#\#}P < .01$ was considered significantly difference compared with normal group compared with model group

forming the structural basis for talin to promote the aggregation of integrins into clusters. Integrin activation reaction is mainly through the talin-FERM structure domain F3 combination of integrin beta of intracellular segment to complete and can be seen from the protein structure of talin can join on integrin beta subunits, joins the actin, and can be directly or indirectly connected with adhesion molecules protein, thus cell adhesion formation, the dynamical process of the collapse of the core protein. siRNA interference with talin expression significantly inhibited the activation of integrin activation,[20] but after the expression of talin is restored, integrin can be activated again.[21] In vivo, there are two states of talin: self-inhibition and activation. In the self-inhibition state, the integrin-binding site F3 in the head of talin binds to the tail segment talin-R9, and the integrin cannot be activated.[22] Studies have shown that lack of talin leads to non-activation of integrins, and talin is also involved in various integrin-mediated responses such as cell deformation, growth, differentiation, and migration in worms and drosophila.[23,24] At present, the regulation mechanism of activation and self-inhibition of talin is not very clear, which needs a further study. The study on how talin self-inhibits and activates, as well as the mechanism of tumor cell invasion and metastasis induced by its synergistic action with related molecules, is helpful to provide new research directions and ideas for the invasion and metastasis mechanism of clinical gastric cancer.

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