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Trop2 promotes vimentin expression and induces epithelial stromal transformation, invasion and metastasis of gastric cancer cells by regulating β-Catenin

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ABSTRACT

One of the most critical biological characteristics of gastric cancer (GC) is invasion and metastasis, which is also the main factor of recurrence and drug resistance. Epithelial intermediate transformation is a biological process. Epithelial cells lose the ability to exercise their epithelial characteristics while acquiring parental characteristics. Malignant epithelial cancer cells lose their connectivity and polarity through the EMT process, change cell morphology and enhance their migration ability, so as to gain the ability of invasion and variation. In this paper, we proposed that trop2 can promote vimentin expression by regulating β - Catenin to induce the transformation and metastasis of gastric cancer cells. In this study, a control group experiment was set up to construct mkn45tr and nci-n87tr resistant cell lines. The results showed that the resistance index (RI) of mkn45tr was 31.33, $P < 0.01$; the resistance index (RI) of nci-n87tr was 108.23, $P < 0.01$. The results show that the drug resistance of gastric cancer cells will become stronger with the change of time.

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Introduction

Gastric cancer (GC) is the most common gastrointestinal cancer in China. 80% of patients with GC are in the advanced stage when they are diagnosed. The effect of surgery and chemotherapy on the overall survival improvement of patients in the advanced stage is not obvious. The aging population, environmental problems and lifestyle have made the mortality rate of gastric cancer slow down in China. As we all know, tumor cells have a strong ability to invade the human body and spread, which is also the main cause of death in tumor patients. Therefore, the study of trop2 promoting vimentin expression and inducing epithelial-stromal transformation and metastasis of GC cells by regulating β - Catenin has great medical feasibility (1,2).

GC is a kind of malignant tumor, and it's common in the world, and its incidence rate and mortality rate are fourth in the world. At present, the treatment of GC is mainly surgery and chemotherapy, combined with biological targeting and immunotherapy and other comprehensive treatment measures, which improve the clinical efficacy to a certain extent, but recurrence and metastasis are still the main reasons for poor prognosis and high mortality of GC patients. Zhao L discussed the effect of catenin and cytokine inhibitors on the migration of gastric cancer cells (1-2). He used recombinant adenovirus to transfect gastric cancer cells and detected protein levels by western blotting (3-4). At the same time, he also used flow cytometry and migration method to detect the effect of catenin on the migration cycle of GC cells (5-6). The results show that cytokines can accelerate the proliferation of GC cells (7-9). This study provides a certain reference value for the clinical prevention and treatment of GC, but its practicability still needs to be confirmed.

MiRNA has a huge impact on the occurrence of GC, like miR-7, mir-217 and miR-335. Wnt's regulation of β - Catenin degradation is the key to tumor development. Tao W found that the acidification of amino-terminal serine and threonine phosphates can lead to the rapid degradation of catenin, and is the result of their joint behavior (10-11). In addition, he also mentioned another kinase, which is a substance necessary for the acidification of catenin (12-13). Moreover, this kinase can inhibit the degradation and acidification of catenin and leads to abnormal embryogenesis related to excessive Wnt/β-Catenin signals (14-15). His research revealed the different functions and steps of phosphorylation of β - Catenin and determined that CKI α is a component of Wnt/β - Catenin signal transduction, which has certain significance for the pathogenesis and treatment of human cancer and diabetes (16-17). The findings of this study provide a certain factual basis for the treatment of GC and a good research direction, but the research still needs to be further discussed.

Gastric cancer has always been a hidden danger that affects people's healthy life. In order to find an effective treatment, this paper studies the epithelial-stromal transformation and metastasis of GC cells. In this paper, by setting up experiments, the drug resistance and morphological changes of GC cells were specifically observed, and what effects the drugs would have on the morphological changes of GC cells. The data results showed that the drug resistance index (RI) of MKN45TR was 31.33, while that of NCI-N87TR was 108.23, which indicated that the drug

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resistance of gastric cancer cells would become stronger with the change of time (18-23).

Gastric cancer

The occurrence of GC may lead to the occurrence of epigastric pain and may lead to the occurrence of digestive tract malignant tumors. The occurrence of gastric cancer must be detected early, controlled early, and reasonable rest must be paid attention to avoid the occurrence of disease aggravation (18). According to statistics, less than one-third of gastric cancer patients are found and diagnosed within three months, that is to say, only some patients may receive treatment in the early stage of GC, most patients are in the middle and late stage, and the curative effect is not ideal. So, what is the initial symptom of gastric cancer? Although the initial symptoms of gastric cancer are not obvious, they can also be detected. More than 80% of gastric cancer patients will feel upper abdominal pain, and about one-third of them will have abdominal discomfort, stomach swelling, loss of appetite, acid reflux and other symptoms, some of them have no gastrointestinal symptoms, only performance (19-20). Gastric cancer is cancer with a high incidence rate. The incidence rate of GC is higher in men over 50 years old, and more in men than women (21-23). Gastric cancer can occur in any part of the stomach, so we should be vigilant. Once we find that the stomach is uncomfortable, we should go to see a doctor as soon as possible, so as to find and treat it early. Because cancer can be divided into early and late stages, early gastric cancer can be improved by drug treatment and reasonable rest; if it is found at a late stage, chemotherapy and other treatment methods are needed, and the effect is not obvious (24). The incidence rate of gastric cancer in China shows a trend of a slow decline, but the rate of decline is very slow. The incidence rate in rural areas and the areas with a high incidence of GC still stands. The incidence rate of gastric cancer also presents two extremes. On the one hand, these two extremes refer to the rejuvenation of gastric cancer, on the other hand, they refer to the aging of gastric cancer. Take different treatment methods at different stages, the best method is a reasonable diet, and healthy living habits, if there is a problem, early detection and treatment should be done (25).

Epithelial stromal transformation

Lens epithelial cells can form the pseudo foot and transform the morphology of one layer into three-dimensional long-range gel culture. According to later studies, Epithelial-mesenchymal transition can have important effects on embryonic development, treatment of chronic inflammation and reorganization of skin tissue. EMT is related to epithelial cell malignancy. The main epithelial tumor cells migrate to different places through EMT cambium cells, thus forming tumor metastasis from epithelial cells. The phenomenon of epithelial-stromal transformation (EMT) exists in various human epithelial malignant tumors, which is manifested in the transformation of epithelial cells from epithelial phenotype to intermediate perfusion phenotype, obtaining some characteristics of intermediate infusion cells. For example, the down-regulation or loss of some indicators of epithelial cells, and the up-regulation of intermediate fluid indicators may lead to an increase in cell mobility, intervention and dynamic characteristics of metastasis. In solid tumors, due to the abnormal structure and function of tumor blood vessels and the rapid proliferation of cancer cells, the oxygen consumption of cancer cells increases, resulting in hypoxia of cancer cells. Hypoxia is one of the important characteristics of microenvironment tumors, which has a huge impact on the proliferation, invasion and metastasis of tumor cells (26). GC is a common solid tumor, at the same time, there is anoxia of cancer cells (27).

Vimentin summary

Intermediate fibers (IF) constitute the skeleton structure of eukaryotic cells and provide stability for cells. It is a kind of strong polymer composed of fibers. These cytoskeleton proteins show interesting molecular diversity and tissue specificity. Vimentin is generally present in stromal cells such as endothelial cells. It was first found in chicken embryo fibroblasts (28). The structure of vimentin is almost fixed from mice, and dogs to higher vertebrates such as apes, showing dynamic expression patterns in different cell types and development stages. The homology of human and mouse vimentin genes is 91.7%, which encodes the same amino acids as simian and canine genes, and the homology is close to 100%. Two dimers are staggered and arranged in reverse parallel to form a tetramer, which is the basic subunit of if. Vimentin can balance itself without the help of other factors (29).

Vimentin and cell adhesion, migration and signaling

IF has traditionally been thought to act only on the mechanical stability of cells, but recently it has been considered a regulator of signal transduction. Vimentin can bind to many other proteins and appears as an organizer of cell adhesion, movement, and signaling. The depletion of vimentin can lead to the dysfunction of the vascular endothelial barrier and leukocyte overflow. The decrease of blood flow-induced dilation in the process of arterial remodeling in the mice lacking vimentin indicates that vimentin plays an important physiological role in regulating tissue differentiation (30).

Materials and Methods

Materials

Cell lines MKLN45 and NCI-N87 were purchased by the laboratory. The main consumables for cell culture are serum, 1640 medium, trypsin; 6, 24, 96 well plates, cell culture bottles, dishes, coked culture dishes, and trastuzumab. The main equipment is a cell incubator.

Methods

Manufacturing of drug-resistant cell lines and cell resuscitation: 1) Heating hydro-fluoride at 37 ℃; 2) Irradiating the clean workbench with ultraviolet for 30 minutes; 3) Placing the sterilized centrifuge tube and straw on the very clean workbench and culture bottle; 4) Remove the cooling pipe; 5) Quickly pull off the plug, quickly put the cooling pipe into the heating water bath, quickly defrost and continue to move so that the liquid in the pipe melts rapidly when there is still thawing in the cooling pipe, take it out; 6) Wipe the outer wall of the cooling pipe with cotton alcohol, and then put it on a very clean table. When stirring the centrifuge tube, add the heated medium drop into the tube. The amount of medium added must be greater than 10ml;7) Focus, focus on 800. A, stay in a low-

speed starting center for five minutes, and then recover the cells with the medium LM; 8) Put the cell suspension into the dish, and put it into the 37-c incubator containing CO2.

Sample handling

According to the staining intensity score of tumor cells, the staining results were 0 Non-staining, 1 light brown, 2 Brown and 3 dark brown. 2 and 3 scores were used as the over-expression standard.

Collect cell protein

Take a bottle of cultured cells in good condition, wash them with PBS solution three times, add LML PBS, scrape the cells off; collect the cells with ooorpmx centrifugation for 5min, wash the cells with PBS buffer solution three times, pour out PBS; add 0.5ml protein lysate (including 24pmsf); split the cells on ice for 30min; centrifuge them at 4 ° C, 12000 rpm for 30min, and transfer the supernatant to another clean 200ul centrifuge tube.

Statistical analysis

Spss19.0 statistical software package was used for statistical analysis. The objective was to compare the correlation between protein expression and clinicopathological parameters by chi-square test, Mann-Whitney test and Bonferroni correction. When P is less than 0.05, it means that the statistical difference is more significant.

Results

Construction of drug-resistant cell line

The sensitive cells were treated with trastuzumab 0, 45, 95, 200, 420, 800, 1500, 2000, 3000 and 3500ug / ml for 48h to detect IC50. The proliferation rate = (mean value of 0d value in other time points / mean value of 0d value in 0h-1) X100% (the same sample), inhibition rate = (mean value of OD value in experimental group + mean value of OD value in the control group) ×100% (the same time). RI = resistant cell IC50 / parent cell IC50. We have successfully constructed MKN45TR and NCI-N87TR resistant cell lines, and the drug resistance index is shown in Table 1. According to Table 1, the drug resistance index (RI) of MKN45TR = 31.33, $P < 0.01$, and that of NCI-N87TR = 108.23, $P < 0.01$. The drug resistance regression curves of the two cells are shown in Figure 1 and Figure 2.

It can be seen from Figure 1 and Figure 2 that the drug resistance of the two cells will become more resistant with the change of time. The drug resistance of nkn45 is stronger than that of MKN45TR, and the drug resistance of NCI-N87 is also stronger than that of nci-n87tr. The drug resistance of nkn45 and NCI-N87 changed relatively steadily with time, and the drug resistance of MKN45TR and NCI-N87 changed significantly with time.

Table 1. IC50 calculation of drug sensitivity of MKN45 / TR and NCI-N87 / Tr cells.

Cell	IC50(ug/ml)	RI	P
MKN45	3168.67	33.13	<0.01
MKN45TR	99250.80		
NCI-N87	4484.23	108.23	<0.01
NCI-N87TR	485457.05		

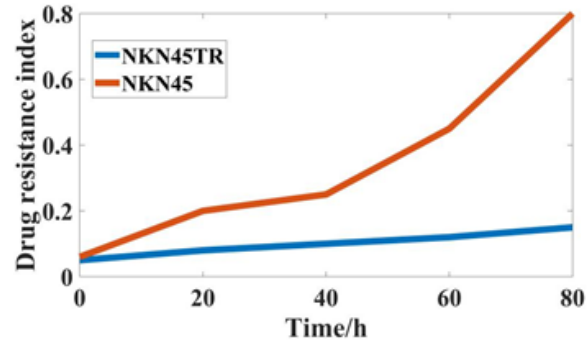


Figure 1. IC50 regression curve of MKN45TR drug sensitivity.

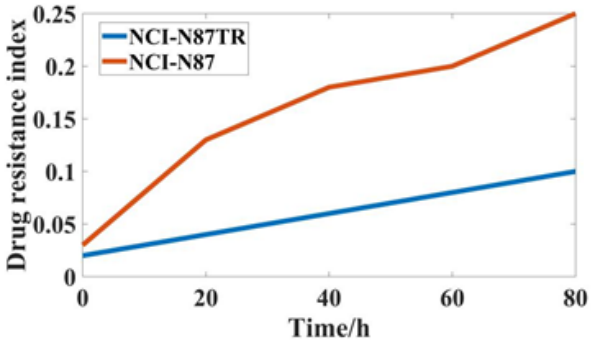
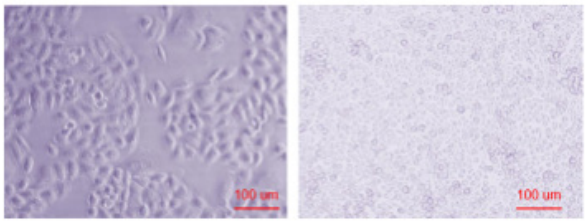


Figure 2. IC50 regression curve of NCI-N87TR drug sensitivity.



(a) MKN45 (b)NCI-N87

Figure 3. Morphological changes of MKN45 / NCI-N87 cells before and after drug resistance.

Morphological changes of gastric cancer cell line trastuzumab before and after drug resistance

After resistance to trastuzumab of HER2-positive GC cell lines MKN45 and NCI-N87, the results are shown in Figure 3.

From the morphological observation in Figure 3, we found that the morphology of the drug-resistant cells changed from epithelioid cells of the sensitive strains to interstitial cells. The data results showed that the cells were elongated, the connections between cells disappeared, and the distribution of cells was loose.

Effect of gastric cancer cell migration

To determine the specific role of PRMT1 in the migration of gastric cancer cells, we used Transwell migration assays. The data results show that when the amount of PRMT1 decreases, the amount of migration cells in the control group was 257 ± 18 and 360 ± 24, respectively, while that in the sh-EGFP group was 52 ± 15 and 88 ± 20, respectively, as shown in Figure 4.

It can be seen from the above experiments that the

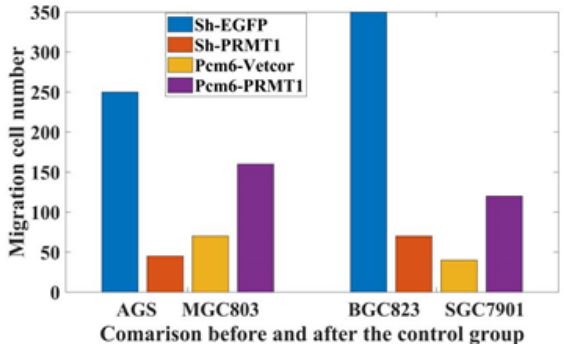


Figure 4. Effect on the migration of gastric cancer cells.

migration ability of AGS and MGC803 Cells decreased significantly after PRMT1 was down-regulated, and the difference was statistically significant. At the same time, the migration ability of BGC823 and SGC7901 cells increased significantly after upregulating the expression of PRMT1, and the difference was statistically significant.

Discussion

As we all know, gastric cancer is a common clinical gastric cancer (19,20). When gastric cancer forms, the local tumor volume increases, which is easy to affect health (21,22). It is found that gastric cancer, a malignant tumor, should be treated symptomatically and controlled reasonably. Otherwise, gastric cancer will continue to worsen and develop to a later stage, which is very difficult to cure and may threaten the life and health of patients. Therefore, it is very important to study the treatment of gastric cancer (22,23). In this paper, trop2 promotes vimentin expression and induces the transformation, invasion and metastasis of GC cells by regulating β - Catenin.

In this study, based on the previous research of the research group, the poorly differentiated human GC MKN-45 cell line was selected, and the effect of vimentin on the epithelial-mesenchymal transition was deeply studied. The data results showed that the drug resistance of cells would become more resistant with the increase of time. In the treatment of gastric cancer, we need to find a new way to treat it, we can't rely on drugs.

Trophoblast-2 is the surface antigen of the human trophoblast. Because of its high expression in a variety of solid tumors, it has become a new target for researchers to develop antibody-coupled drugs. In this paper, trop2 promotes vimentin expression by regulating β - Catenin to induce the epithelial-mesenchymal transformation of GC cells. The changes in GC cells and drug resistance were observed in the control group.

Conflict of interest

The authors declare no conflict of interest.

Data availability statement

The data used to support the findings of the research are included in this article.

Ethical approval and informed consent

This study was approved by the Affiliated Hospital of Youjiang Medical University for Nationalities ethics committee.

Author's contributions

(I) Conception and design: Yixia Yin (II) Administrative support: Yixia Yin, Huadong Huang; (III) Provision of study materials or patients: Yixia Yin; (IV) Collection and assembly of data: Shougao He, Yueqiu Qin, Tingzhuang Yi; (V) Data analysis and interpretation: Gaoyu Hu, Yijuan Yin, Zansong Huang, (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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