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The Transcriptional Regulation Mechanism of Transgelin Gene in Colon Cancer Cells

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ABSTRACT

The objective of this study was to explore the transcriptional regulation mechanism of transgelin gene in colon cancer cells. HT29 cells were randomly divided into three groups: CON group, TGT group and DGT group. The transgelin gene was sub-cultured in the control (CON) group, transfected in the TGT (cells transfected with tranglin gene) group, and knocked out in the DGT (cells in which tranglin gene has been knowcked out) group. The protein expression and the gene expression were measured using Western blotting and RT-QPCR, respectively. We found that cell proliferation rates in CON, TGT and DGT groups showed that cells were transfected with trangelin gene, the cell proliferation rate died down, significantly lower than that in CON and DGT groups ($p<0.05$). Cells migration and invasion results in all groups showed that after cells were transfected with trangelin gene, the number of migrated cells decreased significantly lower than in CON and DGT groups ($p<0.05$). Cells invasion results showed that after cells were transfected with tranglin gene, the number of invasion cells decreased significantly lower than that in CON and DGT groups ($p<0.05$). RT-PCR results showed that the transgelin gene in both CON and TGT groups was significantly increased, much higher than that in the CON group. The EST-1 gene expression in the TGT group was significantly reduced, lower than that in the CON group. WB results showed that the transgelin gene in ERK, AKT, MMP and VEGF proteins in CON and TGT groups was subjected to low expression, whereas in the DGT group it was higher than that in the CON group ($P<0.05$). It is concluded that transgelin gene can block the transcription of HT29 cells in colon cancer, thus affecting the normal proliferation and differentiation of cancer cells.

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Authors' Contribution
QZ and YY selected samples. MY and JC compared the experimental results. MT made further medical analysis. All authors conducted the experiments and analysed the results. All authors discussed the results and wrote the manuscript.

Key words
Transcriptional regulation, Colon cancer, Transgelin gene

INTRODUCTION

Cancer is a malignant tumor of epithelial origin. Worldwide confirmed cancer patients have hit hundreds of millions (Shinji *et al*., 2018; Bai *et al*., 2018), and cancer features a high mortality rate and a low cure rate (Matsuo *et al*., 2018; Wan *et al*., 2018; Etter *et al*., 2018). Colorectal cancer (CRC) is the third most common malignancy diagnosed in the world. An estimated 945,000 new cases of CRC are diagnosed, and about 492,000 deaths from cancer occur each year (Siegel *et al*., 2017). Colorectal cancer is a silent malignancy that affects people who may have no significant symptoms such as bleeding or abdominal pain, and worse, malignancy when it is diagnosed that the tumor is growing so large that it is difficult to treat (Yu *et al*., 2017). However, incidence and mortality have been declining over the past two decades, largely due to attention to screening, early detection, and the introduction of new and effective therapies to control metastasis (Arnold *et al*., 2017). The survival of a patient with colon cancer is affected by the progression of the disease. Most colon cancers start with the unbalanced, noncancerous growth of a polyp, and if these polyps or polyps come out of the bowel, cancer may be prevented (Wolf *et al*., 2018). That is why screening for this malignancy is effective in both reducing the incidence and mortality rate of this cancer. In a taxonomy consortium in 2015, colorectal tumors were classified into four consensus molecular subgroups (CMSs), each with its characteristics (Biswas *et al*., 2016). CMS1 tumors are often characterized by the rhizome-aerobic instability phenotype (MSI) and by activated immune pathways (Zhou *et al*., 2016). This group has the best response to therapies that rely on safety inspections. The activity of Metalloproteinase Matrix (MMP) genes is also evident in this group, and on the other hand, the presence of multiple mutations in these tumors causes the production of new antigens (Sun *et al*., 2017). CMS2 tumors are characterized by epithelial differentiation and activation of the WNT and MYC molecular pathways. CMS3 tumors are characterized by a high prevalence of mutations in the KRAS gene and the activity of metabolic pathways. CMS3 tumors also have high chromosomal instability (CIN) (Meng *et al*., 2017). β-TGF belongs to a large family of structural regulatory

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proteins containing more than 40 proteins and has been discovered as a potent inducer of renal fibroblast growth. Because of its prominent role in regulating cell growth, differentiation, and migration, β-TGF has been identified as an essential factor in cancer progression (Lew *et al*., 2020). Most β-TGF responses include CTGF stimulation at some levels such as ECM component stimulation, fibroblast promotion, osteoblasts, astrocytes, wound healing, and fibrosis. Often referred to as SM22, the transgelin gene (TAGLN) is a TGFβ-inducible gene that is highly expressed in most body tissues, including the bladder, colon, uterus, and prostate (Xu *et al*., 2016). The actin/gel-binding protein TAGLN, a member of the calponin family, is found in the cytoplasm and is expressed in several cells, including fibroblasts, endothelial cells, and immune cells (Lin and Lew, 2019). As an actin cross-linked protein, by growing the production of podosomes and in many biological processes related to the growth of cancer, such as proliferation, differentiation, migration, invasion, and apoptosis, TAGLN participates in cell motility. TAGLN-deficient mice are fertile and develop normally, but they decrease the contraction of smooth muscles and have key differences in actin filament distribution and skeletal organization (Dvorakova *et al*., 2020). The expression and function of TAGLN in cancer biology depends upon the type and stage of cancer, similar to TGFβ, so the role of TAGLN is controversial in CRC biology (Munakata *et al*., 2018).

Due to the high prevalence of colorectal cancer the present study aimed to explore the transcriptional regulation mechanism of transgelin gene in colon cancer cells.

MATERIALS AND METHODS

Experimental subject

HT29 cells in the U.S. ATCC Cell Lines were purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai). Cells were divided into three groups: CON group (n=16), TGT group (n=16) and DGT group, each has 16 culture dishes. Cells were cultured without any processing in the CON group, cultured after transfecting the transgelin gene in the TGT group, and cultured after knocking out the transgelin gene in the DGT group.

Culture of HT29

The culture temperature was 37 °C and the CO_2 concentration was 5%. The culture solution was DMEM medium containing 10% fetal bovine serum with double-antibody added. Under the microscope, the cells were sub-cultured when they reached 80%-90% of the visual field. They were sub-cultured about once every two days. In the process of sub-culture, the culture medium was removed and washed by Hanks. After that, cells were digested with 1 mL of trypsin for 1 min and washed again using the cell culture medium. Then 1 mL of culture medium was added to aspirate off the adherent cells, which were placed in the culture flask by the way that one cell transmitted to two generations.

Transfection of HT29 with transgelin

Competent cells were added with plasmids and conjugate products, then rapidly bathed at 45°C after being placed on the ice and later placed on the ice again. Finally, after culturing on the culture plate containing antibiotics overnight, plasmids were extracted as instructed and the agarose gel electrophoresis (AGE) was used to test the DNA expression, including purity and the amount of expression. Transfection of plasmids: well-grown cells were inoculated in the culture place, with each hole added by a plasmid-containing serum-free medium. After being added with the transfection reagent, these cells were placed in the incubator for culture and shaken every 10 mins. The culture medium was replaced after 6-8 h. Amplification of the target gene: after extracting RNA, PCR amplification was done to obtain cDNA, then primers were added for PCR amplification with cDNA as the template. Upon digestion with restriction enzyme after amplification, siRNA was transfected.

HT29 invasion and migration assay

Invasion assay: Cells were added to the upper chamber of Transwell, after which colon cancer cells passing through the matrix gel-coated lower chamber were stained with eosin dye to microscopically count them. Migration assay: Cells were added to the upper chamber of Transwell microscopically count them in the lower chamber. Each group conducted 6 parallel assays.

RT-PCR

50×TAE was diluted into 1×TAE solution as a solvent and 0.5 g of agarose was weighed to add to the

Table I. Transgelin, EST-1 and β-actin Primer.

Gene name	Primer sequence 5' → 3'		Length (bp)
Transgelin	F: ACTAAAGCTGGTTGGTCCCGT	R: GCTAGCTCGGCTC-GCCTGCCC	111
EST-1	F: GCTCGTAGACCGTCTCCCGTC	R: GCGCTAGCCGCGTTTAATAATT	102
β-actin	F: CTACGCTAGCTGTAATCGATC	R: GATGCGATCGCTGATCGCTAGC	127

1×TAE solution. After heating in a boiling water bath, 4 μL of nucleic acid dye was added and shaken to mix. Finally, the agarose gel was placed horizontally in the electrophoresis bath, and 5 μL of DNA Maker and target gene PCR amplification product were added in order and photographed (Table I).

Western blotting

Protein was extracted and the NC membrane was placed into a plate dish. Then skimmed milk powder was added to the container and sealed in the dark on a shaker for 0.5-1.5 h. After discarding the milk powder, the NC membrane was taken out in TBST for washing for three times. Then primary antibody was added for incubation overnight at 4°C. On the next day, the NC membrane was taken out in TBST solution for washing for four times, after which secondary antibody was added for incubation. Then the prepared color development solution (luminous solution prepared based on 1:1 ratio of liquid a and liquid b when needed) was dropped over the NC membrane. 1 mL of color development solution was added slowly from right to left before being photographed and scanned in the dark room.

Statistical analysis

In this study, all data were processed by SPSS20.0 statistical analysis software (IBM Corporation, USA). Mean ± standard deviation was used to express the measurement data. One-way ANOVA or repeated measures ANOVA was employed for the comparison among all groups and LSD-t test was used for the comparison between two groups. The count data were expressed in percentages (%) and comparisons among groups were analyzed by χ^2. P<0.05 represents a statistically significant difference.

RESULTS AND DISCUSSION

Cell proliferation rates

After the transfection of transgelin, as culture time extended, the cell proliferation rate decreased and finally to 1.05±0.32, lower than that in CON and DGT groups with the difference subject to statistical significance (P<0.05). After the knockdown of transgelin, the cell proliferation rate increased, higher than that in the CON group. The difference in cell proliferation rate between the TGT group and CON and DGT groups has statistical significance (*P*<0.05). This indicates that transgelin gene can block the proliferation and differentiation of HT29 in colon cancer, thus inhibiting the development of cancer cells (Table II).

HT29 migration and invasion capabilities

After the transfection of transgelin, the number of migrated cells decreased and finally to 110±14, lower than that in CON and DGT groups with the difference subject to statistical significance (P<0.05). After the knockdown of transgelin, the number of migrated cells increased, higher than that in the CON group with the difference subject to statistical significance (P<0.05). Cell invasion results showed that after the transfection of transgelin, the number of invaded cells decreased, lower than that in CON and DGT groups with the difference subject to statistical significance (P<0.05). After the knockdown of transgelin, the number of migrated cells increased, higher than that in the CON group with the difference subject to statistical significance (P<0.05). Migration and invasion experiments showed that transgelin can inhibit the activity of HT29 in colon cancer (Table III).

Table II. Cell proliferation rates (%) in colorectal cancer cells.

Group	12 h (n=16)	24 h (n=16)	48 h (n=16)
HC	2.53±1.63**	1.64±0.85**	1.05±0.32**
NOR	12.37±2.64	14.45±2.17	17.53±2.74
DHC	14.73±2.46**	19.83±2.84**	25.75±4.62**
F value	15.21	12.40	22.75
P value	0.002	0.007	0.003

HC, Transfection group; NOR, Blank group; DHC, Knockout group. Note: Compared with the NOR group, **P* <0.05.

Table III. Cell migration and invasion in CRC cells.

Group	Migration (%)	Invasion (%)
HC	110.05±14.63	289.62±21.30
NOR	252.29±12.75**	885.31±39.29**
DHC	693.25±15.17**	1250.23±43*
F value	28.134	36.279
P value	0.007	0.002

Note: *Compared with the NOR group, *P* <0.05; # compared with the NOR group, *P* <0.01. For abbreviation see Table II.

Expression of transgelin and EST-1

EST-1 is an oncogene that plays an important role in apoptosis and transcription of colon cancer cells. In this experiment, after the cells were collected by centrifugation and RNA in the cells was extracted from the kit. Compared with that in the DGT group, the amount of expression of transgelin in CON and TGT groups increased, where that in the TGT group was higher than that in the CON group. Compared with that in the DGT group, the amount of expression of EST-1 in CON and TGT groups decreased, where that in the TGT group was lower than that in the CON group. This proves not only the success of transgelin

in transfection and repression but also confirms that upregulation of tran sgelin can inhibit the transcriptional translation of the important gene EST-1, thus affecting the proliferation and differentiation and migration of colon cancer cells (Table IV, Fig. 1).

Expression of AKT and ERK in colon cancer cells

AKT and ERK are key pathway proteins for cancer cell proliferation. AKT and ERK expression results in three groups showed that compared with that in the DGT group, the expression of AKT and ERK was low in CON and TGT groups, where that in the DGT group was higher than that in the CON group, with statistical significance (P<0.05) (Table V, Fig. 2).

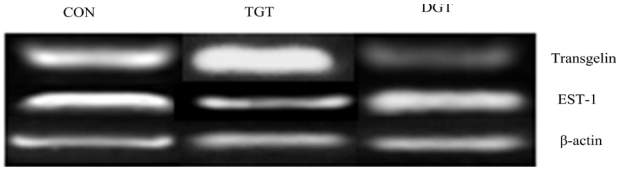

Fig. 1. Expressions of TP53 and RAI-1 in three groups with RT-QPCR.

Table IV. Degree of expression of transgelin and EST-1 in different colon cancer cells.

Group	Transgelin (Gray value)	EST-1 (Gray value)
CON	3.24±0.64	3.79±0.84
TGT	6.37±0.58**	2.18±0.53**
DGT	1.56±0.36**	3.35±0.71**
F value	9.654	8.725
P value	0.007	0.002

CON, control; TGT, cells transfected with trangelin gene; DGT, cells in which trangelin gene has been knocked out. Note: *Compared with the NOR group, P <0.05; # compared with the NOR group, P <0.01. (CON) group, transfected in the TGT () group

Table V. Degree of expression of AKT and ERK in different colon cancer cells.

Group	n	AKT (Gray value)	ERK (Gray value)
CON	10	1.52±0.21	2.27±0.48
TGT	10	0.93±0.06**	1.09±0.14**
DGT	10	3.11±0.26**	4.14±0.64**
F value		10.446	8.947
P value		0.005	0.005

Note: *Compared with the NOR group, P <0.05; # compared with the NOR group, P <0.01. For abbreviation see Table IV.

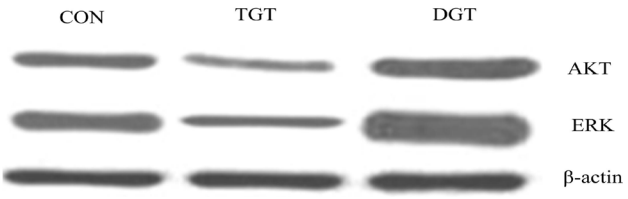

Fig. 2. Expressions of AKT and ERK in three different types of colon cancer cells with western blot. For abbreviations, see Table IV.

Expression of MMP and VEGF in CRC

MMP and VEGF are key proteins that control colon cancer cells. WB results showed that compared with that in the DGT group, the expression of MMP and VEGF was low in CON and TGT groups, where that in the DGT group was higher than that in the CON group, with statistical significance (P<0.05). This confirms that upregulation of transgelin can inhibit the expression of MMP and VEGF and the transcriptional translation of cancer cells, thus affecting their proliferation and differentiation (Table VI, Fig. 3).

Table VI. Expression of MMP and VEGF in different colon cancer cells.

Group	n	MMP (Gray value)	VEGF (Gray value)
CON	10	2.94±0.31	2.95±0.47
TGT	10	0.74±0.06**	1.32±0.15**
DGT	10	3.85±0.64**	3.75±0.54**
F value		11.065	9.732
P value		0.004	0.002

Note: *Compared with the NOR group, P <0.05; # compared with the NOR group, P <0.01. For abbreviation see Table IV.

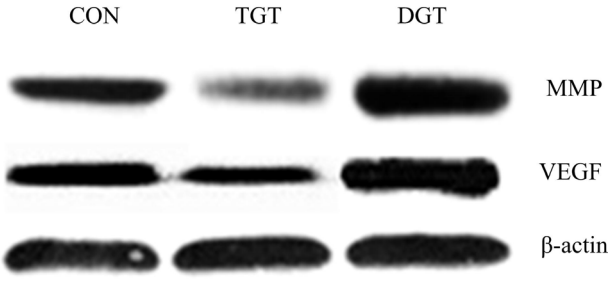

Fig. 3. Expressions of MMP and VEGF in three different types colon cancer cells in three groups with western blot. For abbreviations, see Table IV.

DISCUSSION

Colon cancer is a malignant tumor commonly found in the colon, with a higher prevalence in men than in women. The incidence of colon cancer in China is increasing year by year (Jain *et al*., 2018), and as reported, its development is associated with high intake of fat and low intake of fiber, with clinical symptoms mainly including low fever, fatigue, anemia, edema and weight loss. The progression of colon cancer to a younger age makes the treatment of colon cancer urgent. Finding suitable targets has also become a hotspot of current study.

Cancer features rapid proliferation and strong invasion and migration, making the latter a vital phenotypic index to judge the viability of cancer cells. It has been reported that the severer the degree of cancer in cancer model mice, the stronger the migration and invasion of cancer cells (Tian and Yuan, 2018). In this experiment, Transwell results showed that the transfection of transgelin, the number of invaded cells lessened, lower than that in CON and DGT groups, and that in the DGT group was more than that in the CON group. Cell invasion results showed that after the transfection of transgelin, the number of invaded cells decreased, lower than that in CON and DGT groups, indicating that transgelin can inhibit the activity of HT29.

EST-1 is an important regulatory transcriptional gene in cancer that directly controls the expression of important downstream proteins in cancer cells (Hou *et al*., 2017). Massive coverage has confirmed that EST-1 can regulate the pathway of cancer cells and cause them to proliferate and differentiate. Its existence may directly affect the growth of cancer cells. Studies on EST-1 have been reported many times, but the relationship between transgelin and EST-1 in colon cancer cells has not been covered. In this experiment, results showed that compared with that in the DGT group, EST-1 in CON and TGT groups decreased, where that in the TGT group was lower than that in the CON group. This proves that transgelin may inhibit the transcriptional translation of EST-1, thus affecting the proliferation and differentiation and migration of colon cancer cells.

The AKT pathway is a classic cancer cell apoptosis pathway (Wang *et al*., 2018; Han *et al*., 2018), and when stimulated, cancer cells may directly lead to a decrease in the phosphorylation level of AKT (Tian and Yuan, 2018) and affect the expression of the downstream protein ERK (Pouysségur and Lenormand, 2016, Zheng *et al*., 2017), which in turn affects the expression of the key proteins MMP and VEGF (Xia *et al*., 2016; Liu *et al*., 2017). It is reported that the rise in VEGF content in cancer model mouse serum (Di *et al*., 2016; Cho *et al*., 2019) has not been associated with the AKT cancer passway (Lee *et al*., 2018; Lima *et al*., 2016). In this experiment, results showed that compared with that in the DGT group, the expression of AKT and ERK in CON and TGT groups was low, where that in the DGT group was higher than that in the CON group. Compared with that in the DGT group, the expression of MMP and VEGF in CON and TGT groups was low, where that in the DGT group was higher than that in the CON group. The proliferation rate, migration quantity and invasion quantity in the TGT group were lower than those in CON and DGT groups. Gene and expression results showed that the amount of expression of EST-1 in HT29 in the TGT group was lower than that in the CON and DGT groups. Protein expression results showed that the amounts of expression of AKT, ERK, MMP and VEGF in the TGT group were lower than those in the CON group, where those in the DGT group were higher than those in the CON group. Therefore, it was speculated that transgelin can inhibit the transcription of EST-1 and affect its expression and by affecting the AKT pathway, it can decrease the amounts of expression of ERK, MMP and VEGF, thus inhibiting the invasion and migration of colon cancer cells.

In conclusion, transgelin can block the transcription of HT29 in colon cancer, thus affecting the normal proliferation and differentiation of cancer cells.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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