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Expression of GNAS-AS1 In Rectal Cancer and Its Effects on Proliferation, Migration and Invasion of Rectal Cancer Cells

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Abstract

Objective: To study the molecular mechanism of long non-coding ribonucleic acid (lncRNA) GNAS antisense RNA 1 (GNAS-AS1) in affecting the proliferation, migration and invasion of rectal cancer cells.

Methods: The levels of GNAS-AS1 and micro ribonucleic acid (miR)-34c-5p in rectal cancer tissues, paracancerous tissues and rectal cancer SW1463 cells were determined using qRT-PCR, and Western blotting was conducted to measure the content of p21, E-cadherin and matrix metalloproteinase (MMP)-2 proteins. The cell viability rate was measured using MTT assay, and the number of migrating and invading cells was counted by Transwell assay. The targeting relationship between GNAS-AS1 and miR-34c-5p was verified using dual luciferase reporter assay.

Results: Compared with normal paracancerous tissues, rectal cancer tissues had significantly increased GNSA-AS1 content ($P<0.05$). Inhibiting the expression of GNAS-AS1 suppressed the proliferation, migration and invasion of rectal cancer SW1463 cells, raised the content of p21 and E-cadherin proteins and the percentage of cells in G0 phase and decreased the content of MMP-2 and the percentage of cells in S1 phase. GNAS-AS1 bound to miR-34c-5p in a targeted manner, and their expressions showed a negative correlation. Moreover, repressing the expression of miR-34c-5p weakened the effects of down-regulated GNAS-AS1 expression on the proliferation, migration and invasion of SW1463 cells.

Conclusion: GNAS-AS1 modulates the proliferation, migration and invasion of rectal cancer SW1463 cells by targeting miR-34c-5p.

Key words: rectal cancer, GNAS-AS1, miR-34c-5p, proliferation, migration, invasion.

1. Introduction

The five-year survival of rectal cancer remains poor in each stage despite continuous advancement of diagnostic and treatment technologies, at approximately 53 percent (Khalfallah M.et al., 2017). OR is the main type of clinical rectal cancer treatment, while productive after surgical targeted care provides the primary means of enhancing patients' survivability and quality of life and management of cancer (Benevento I, et al. 2017) (Rödel)C. et al., 2016).

The rectal cancer is abnormally expressed with several long, non-coding ribonucleic acids (lncRNAs), RNAs micro (miRNAs) and messenger RNAs (mRNA), and its progression is modulated via the regulatory network lncRNA-miRNA-mRNA (Li N. et al. 2019). GNAS antisense RNA 1 (GNAS-AS1) is the upregulated expression lncRNA in non-small lung cell carcinogenic (NSCLC) and promotes cancer cell migration and invasion (Li Z. et al., 2020). GNAS-AS1 has high expression and can be involved in cancer growth and progression in tissue colorectal cancer (Zhang Y. et al., 2019). It was expected to use this study TargetScan that

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there

were binding sites Mirr-34c-5p and GNAS-AS1. MiR-34c-5p is known as an important tumor suppression system whose expression is colorectal cancer decreased and associated with cancer cell proliferation, metastasis and apoptosis (Yang S. et al., 2014). GNAS-AS1 and miR-34c-5p, however, are not understood and play a role in rectal cancer. The meaning of GNAS-AS1 in This study has been performed on rectal cancer tissues with the objective to establish an emerging goal for molecular targeted treatment for rectal cancer by explorating the molecular mechanism by which GNAS-AS1 and miR-34c-5p influence proliferation, invasion and migration of SW 1463 cels as research objects.

2. Materials and methods

Materials

The rectal cancer tissues and parancerous tissues (> 5 cm away from the rectal tissues margin) have been chirurgically resected from 23 patients, including 15 males and 8 females, aged 18 to 72, and aged 44.35 + ± 15.68, who were pathologically diagnosed with rectal cancer. There were 3 rectal cancer cases of stage I, 10 cases of stage II rectal cancer, 7 cases of stage III rectal cancer and 3 cases of stage IV rectal cancer according to the eighth version of the TNM rectal cancers staging system. No radiation and chemotherapy was given to all patients before surgery. This research has been carried out with the approval of the hospital's Medical Ethics Committee, and all patients have given their informed consent. ATCC was bought from the human rectal cancer SW1463 cell lines. The Dulbecco Modified Eagle Medium (DMEM) was obtained from Gibco and the Corning, primers, and GNAS-AS1 Interfering Little GNAS-AS1 Interference Plates (miR-34c-5p mimiq) (miR-34c-5p) have been purchased from Corning, primers and GNAS-AS1. Dimethyl sulfoxide (DMSO) and trypsin were obtained from Sigma-Aldrich Co., LLC. Testing device for dual luciferase reporters and transfectation reagents for Lipofectamine 2000 were purchased from Promego, USA, as well as Invitrogen, USA. Total RNA extraction kits, PCR reaction kits, and RT PCR reverse transcription kits were also collected from TaKaRa Biotech (Dalian) Co., Ltd., and Bio-Rad (USA) was offered as a real-time PCR tool.

Methods

Cell culture

Cell cultivated medium has been prepared in 10% SW1463 rectal human cancer cells in a 5% co2 incutatory, accompanied by digestion, subculture + 10% SW1463 high glucose DMEM + 1 1 105% U / L penicillin + 100mg / L streptomycin

Cell transfection

The cells SW1464 were diluted by the cell medium to generate 1-1106 cell / mL of concentration after culture until the logarithmic growth process. The cells were then inoculated on a 200 μL 6-wave plate / well and transfected according to Lipofectamine 2000 instructions when a confluent layer has been created. The well-cropped 6-wave SW1463 cells have been transfeced with Si-NC or si-GNAS-AS1 vector miR-NC or miR-34c-5p and si-GNAS-AS1 or si-GNAS-AS1 + vector SW1463. The original medium has been replaced by a full medium after six hours of community. Cells were harvested and subjected to an RT-PCR (qRT-PCR) for verification at 48 hours after the transfection. After the successful transfection was confirmed, the experiments were performed.

Measurement of GNAS-AS1 and miR-34c-5p expressions by real-time PCR

The transfected SW1463 cells in each group were collected after 48 hours of cultivation and the entire RNAs have been extracted using the package. The concentration and purity have subsequently been calculated. At -80 ° C RNAs have been deposited. Additional deoxyribonucleic acids (cDNAs) had been subsequently Synthesized in accordance with the RT-PCR Kit instructions and procedures as follows: 16 ° C in 30 minutes, 42 ° C in 30 minutes and 72 ° C in 5 minutes. Then the synthesized cDNAs were put at the temperature of 4 ° C for 10 minutes and measured their concentration and purity. They were held at -80 ° C afterwards. Then, the GNAS-AS1 and miR-34c-5p synthesizers were used to obey the instructions of real-time PCR. In conclusion, data have been reviewed using the System 2- bisCt.

Detection of cell viability by MTT assay

The transfected SW1463 cells were collected, trypsinized from each group, and seeded into a 96-well plate in 3 to 103 cells / well concentrations. The MTT assays were performed after cultivation in the 5% CO2 incubator at 37 ° C for 48h, as follows: cells were added in 20 μL (5 mg / mL), and further cultivation was performed for four hours with MTT solution. Every well has been added to solve crystals with 150 μL of DMSO and shaken at room temperature for 5 min with

the supernatant medium discharged.

Subsequently, the absorbance (A) was determined using a microplate reader at 490 nm. The viability rate (%) = $A_{experiment\ group}/A_{control\ group} \times 100\%$.

Detection of cell migration and invasion by Transwell assay

Migration assay: After transfection, the SW-1463 cells of each group have been cultivated overnight in an FBS-containing high-glucose DMEM, up to a logarithmic growth level. The cells were then digested with trypsin and twice washed with PBS and diluted using high glucose DMEM without serum until 1 to 105 cells / mL concentration is observed. With 500 μL of medium containing 10% FBS the cultivation fountains in the Transwell chamber were added, with the upper chamber adding 100 μL in SW1463 cell to the cultivation pools in the lowness chamber. The non-migrating cells in the upper chamber, after the cells were grown at the CO2 incubator for 24 hours, were scorched using cotton swabs while migrant cells were fixed with formaldehyde and stained with violet crystal. The resulting cells were detected and analyzed in five fields in each sample under a microscope. The average was eventually achieved.

Invasion assay: Spewed at -20 ° C, the Matrigel was stripped and tanned in a 4 ° C refrigerator overnight. It was then inserted in the upper Transwell chamber at 50 μL / well and solidified at 37 ° C at a ratio of 1:6 with the use of a serum-free medium at 4 ° C. The following measures were similar to those of the migration exam.

Verification of targeting relationship between GNAS-AS1 and miR-34c-5p by dual luciferase reporter assay

The SW1463 cells have been cultivated and transferred according to the 1.2.2 process. The wild type (WT)-GNAS-AS1 and/or mutants — GNAS-AS1 dual-luciferose vector and miR-NC or miR-34c-5p have been co-transfected. The lysed cells were centrifugated after 48 hours and the supernatant was collected. Renilla luciferase as an internal guide was used for measurement and estimation of the relative behavior of firefly luciferase.

Statistical analysis

All data were expressed as mean ± standard deviation (total ± s) and analyzed statistically using software SPSS19.0. The independent sample t test was used to render intergroup comparisons. Statistically important was found $P < 0.05$.

3. Results

3.1. Expression of GNSA-AS1 in rectal cancer tissue

Rectal cancer tissue has substantially increased GNSA-AS1 ($P<0.05$) in contrast to normal paracancer tissues (Table 1).

3.2. Effects of GNAS-AS1 inhibition on SW1463 cell proliferation

After transfecting si-GANS-AS1, the si-GNAS-AS1 group was displayed as opposed to si-NC group. significantly decreased GNAS-AS1 content and Increased p21 protein content for SW 1463 cells rectal cancer, decreased cell viability, increased cell percentage for G0 and decreases in S-phase, with statistically relevant variations ($P < 0.05$) (Figure 1 and Table 2). These findings show that inhibiting the expression of GNAS-AS1 will stop the growth of SW1463 cells.

Table 1. **Expression of GNSA-AS1 in rectal cancer tissue ($\bar{x}$ ± s, n=23)**

Group	Sample number	GNAS-AS1
Paracancerous tissue	23	1.03±0.05
Rectal cancer tissue	23	3.42±0.07*
t		133.244
P		0.000

*P<0.05 vs. paracancerous tissue group.

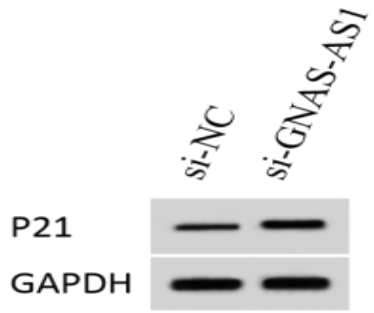

Figure 1. **Expression of P21 protein.**

Table 2. **Effect of GNAS-AS1 inhibition on SW1463 cell proliferation ($\bar{x}$ ± s, n=9)**

Group	GNAS-AS1	G0 (%)	S (%)	G1 (%)	Viability (%)	P21
si-NC	1.00±0.05	34.68±1.59	28.64±1.26	36.68±1.61	100.02±8.26	0.22±0.02
si-GNAS-AS1	0.27±0.03*	45.01±1.83*	17.88±1.01*	37.11±1.52	53.68±5.17*	0.58±0.04*

t	37.558	12.783	19.990	0.5826	14.266	24.150
P	0.000	0.000	0.000	0.5683	0.000	0.000

*P<0.05 vs. si-NC group.

3.3. Effects of GNAS-AS1 inhibition on SW1463 cell migration and invasion

According to the Transwell assay results, si-The GNAS-AS1 group had substantially less SW1643 migrating cells, more protein e-cadherin and less protein-related protein matrix-2 (MMP-2) than si-NC, and statistically significant differences (P<0.05) (Figure 2 and Table 3). GNAS-AS1 group showed substantial reduced results.

GNAS-AS1 targeted miR-34c-5p

The GNAS-AS1 sequences formed a complementary site to miR-34c-5p were predicted by TargetScan (Figure 3). The dual luciferase reporter compares The findings in SW1463 cell fireflies were significantly weaker in miR-34c-5p than in miR-NC group (P<0.05) when the firefly luciferase activity of cells with WT-GNAS-AS1 transfected was relatively low, while the relative firefly luciferase activities of cells transfected with MUT-GNAS-As1 were not significantly changed. It is thus possible to conclude that GNAS-AS1 is targetedly bound to miR-34c-5p. The expression level of miR-34c-5p was found by qRT-PCR to increase significantly in the si-GNAS-AS1 group relative to those in the si-NC group (P<0.05) (Table 5) suggesting a reverse association of the expression of GNAS-AS1 with mirR-34c-5p.

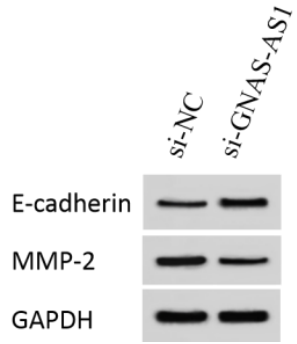

Figure 2: **Expressions of E-cadherin and MMP-2 proteins.**

Table 3: **Effects of GNAS-AS1 inhibition on SW1463 cell migration and invasion ($\bar{x}$ ± s, n=9)**

Group	Number of migrating cells	Number of invasive cells	E-cadherin	MMP-2
si-NC	147±9.16	83±7.21	0.27±0.03	0.81±0.05
si-GNAS-AS1	68±6.35*	41±5.00*	0.72±0.05*	0.29±0.03*
t	21.264	14.361	23.152	26.754
P	0.000	0.000	0.000	0.000

*P<0.05 vs. si-NC group.

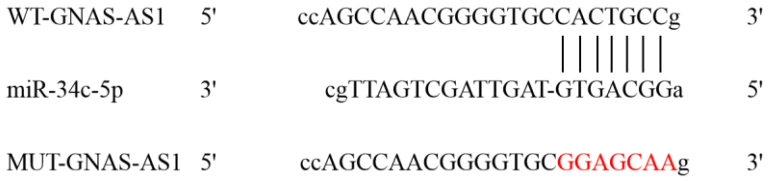

Figure 3: **GNAS-AS1 targeted miR-34c-5p.**

Table 4: **Dual luciferase reporter assay results ($\bar{x}$ ± s, n=9)**

Group	WT-GNAS-AS1	MUT-GNAS-AS1
miR-NC	1.02±0.05	1.00±0.05
miR-34c-5p	0.31±0.03*	0.99±0.05
t	36.529	0.4243
P	0.000	0.6770

*P<0.05 vs. miR-NC group.

Table 5: **GNAS-AS1 regulated expression of miR-34c-5p ($\bar{x}$ ± s, n=3)**

Group	miR-34c-5p
si-NC	1.01±0.06
si-GNAS-AS1	2.68±0.08#
t	50.100
P	0.000

#P<0.05 vs. si-NC group.

3.4. Suppressing miR-34c-5p attenuated the effects of GNAS-AS1 inhibition on SW1463 cell proliferation, migration and invasion

In this study, both GNAS-AS1 and miR-34c-5p were repressed to check whether GNAS-AS1 regulation of miR-34c-5p affects the

proliferation, migration and invasion of SW1463 cells. The si-GNAS-AS1 + anti-miR-NC group displayed a substantially declined miR-34-5p value in comparison with the si-GNAS-AS1 + anti-miR group increased viability rate of cells and number of The cells migrated and invaded, the content of lowered p21 proteins, lowered MMP-2 content, lowered G0
cell percentage and increased s phase s cell percentage and statistically significant differences (P<0.05) (Figure 4, Table 6 and 7). These results show that the repression of the miR-34c-5p expression will weaken the effects on proliferation, migration, and invasion of SW1463 cells of the down-regulate GNAS-AS1 expression.

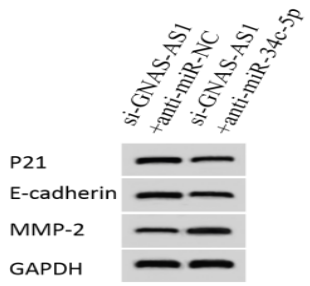

Figure 4: **Expressions of P21, E-cadherin and MMP-2 proteins.**

Table 6: **Suppressing miR-34c-5p attenuated the effects of GNAS-AS1 inhibition on SW1463 cell proliferation ($\bar{x}$ ± s, n=9)**

Group	G0 (%)	S (%)	G1 (%)	Viability (%)	P21
si-GNAS-AS1 + anti-miR-NC	45.03±1.81	17.91±1.04	37.06±1.54	53.73±5.16	0.57±0.04
si-GNAS-AS1 + anti-miR-34c-5p	37.26±1.57*	24.61±1.18*	38.13±1.59	91.07±6.68*	0.28±0.03*
t	9.729	12.779	1.4502	13.271	17.400
P	0.000	0.000	0.1663	0.000	0.000

*P<0.05 vs. si-GNAS-AS1 + anti-miR-NC group.

Table 7: **Suppressing miR-34c-5p attenuated the effects of GNAS-AS1 inhibition on SW1463 cell migration and invasion ($\bar{x}$ ± s, n=9)**

Group	miR-34c-5p	Number of migrating cells	Number of invasive cells	E-cadherin	MMP-2
si-GNAS-AS1 + anti-miR-NC	1.00±0.04	67±6.33	43±5.04	0.71±0.05	0.28±0.03
si-GNAS-AS1 + anti-miR-34c-5p	0.41±0.03*	126±8.15*	72±6.34*	0.35±0.03*	0.64±0.04*
t	35.400	17.152	10.742	18.522	3.568
P	0.000	0.000	0.000	0.000	0.0026

*P<0.05 vs. si-GNAS-AS1 + anti-miR-NC group.

4. Discussion

With the shift in people 's dietary structure, the rates of incidence of rectal cancer are rising and postoperative tumor recurrent and metastasis contribute to poor prognosis and higher patient mortality. Many studies have revealed that lncRNAs, miRNAs, and mRNAs have changed considerably in colorectal cancer development and may serve a possible biomarker for the development of colorectal cancer and as novel markers or targets for clinical diagnosis and treatment (Liu J. et al., 2019).

LncRNA GNAS-AS1 is a single-aller transcript provided by the GNAS promotor, imprinted sites (Griebek V. et al., 2014) in a differentially mechanized region. Expression of NSCLC cells and tissues of GNAS-AS1 is substantially increased, and the overall survival rate for NSCLC patients is negatively correlated. The macrophage polarization of the GNAS-AS1 / miR-4319 / NECAB3 axis shifts to facilitate NSCLC development (Li Z et al, 2020). The most recent research has shown GNAS-AS1 to be overexpressed and linked to Hes1 in order to cause colorectal cancer growth and progression (Zhang Y. et al., 2019).). However, in studies it has still not been clarified whether GNAS-AS1 affects the proliferation, migration and invasion of rectal cancer cell. In this study, the GNAS AS1 content in rectal tissues has been shown to increase significantly in comparison with that in normal paracancerous tissues and the expression of

GNAS AS1 inhibits proliferation , invasion and movement of the cells of the SW1463 system , increasing the protein content of p21 and E-cadherin, and decreasing the cell content in the G0 process and the MMP-2 and The findings above suggest that GNAS-AS1 contributes to the regulation of rectal cancer cell proliferation, invasion and migration..

Furthermore, TargetScan predicts that the sequences miR-34c-5p and GNAS-AS1 contain binding sites, suggesting a potential targeting relationship between the two. Sky-MiR-34c-5p has a downregulated expression in multiple cancers and associated with cancer progression and cancer pain (Gandla J. et al., 2017)? The lowly expressed miR-34c-5p in acute myeloid leukemia (AML has strong ties with poor prognosis of AML patients and adverse reactions (Peng D. et al. 2018). The miR-34c-5p expression of osteosarcoma tissues and cells is considerably less, and themiR-34c-5p over-expression can prevent the proliferation, migration and invasive cells of osteosarcoma, suppress the cycline D1, MMP-2 and MMP-9 protein expressions and promote protein expression of p21 (Wang Y. et al. 2019). Furthermore, miR-34c-5p in laryngeal squamous cell carcinoma is downregulated and can serve as a biomarker for patient prognosis (Re M. et al. 2017). MIR-34C-5p expression is diminished by methylation of its promoter in colorectal cancer, and the methylatedmiR-34c-5p inhibit colorectal cancer metastase and EMT repression by targeting the SATB 2 (Gu J. et al., 2018). The dual luciferase reporter test discovers in this analysis that GNAS-AS1 was targeted and associated with miR-34c-5p with their signals. In addition, the effects of inhibiting GNAS-AS1 expression on the proliferation, migration and invasion of SW1463-cells were weakened by inhibition of miR-34c-5p, suggesting indirectly that GNAS-AS1 controls migration and migration of Sw 1463-cells via miR-34c-5p targeted.

In **Conclusion**, the lnc RNa GNAS-AS1 targets miR-34c-5p in rectal cancer SW1463 cells to modulate its spread, migration, and invasion, and is upregulated in rectal cancer tissues. LncRNA GNAS-AS1 can therefore serve as a possible rectal cancer molecular target.

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