miR-22 suppresses cell proliferation, invasion and migration invasion in human colon cancer cell based on targeting NLRP3

Jinchun Cong
Shengjing Hospital of China Medical University

Jian Gong
Shenyang Pharmaceutical University

Chuanjia Yang
Shenyang Pharmaceutical University

Zhixiu Xia
Shengjing Hospital of China Medical University

Hong Zhang (✉ zanghong7919@outlook.com)
Shengjing Hospital of China Medical University

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Abstract

Background

To investigate the effect of the cell proliferation, migration and invasion in human colon cancer cells which based on the mechanism of miR-22 targeting NLR family pyrin domain containing3 (NLRP3)

Methods

Human colonic epithelial cells (HCoEpiC) and human colon cancer cell lines (HCT116, HCT8, HT29, LS174T, LOVO, SW480) were prepared for the present study. The HCT116 cells were divided into 2 parts with different processes, and each part was randomly divided into different groups (part1: BC, NC1, miR-22, NC2, si-miR and part2: BC, miR-22, NC3, si-NLRP3, miR + NC4, miR + NLRP3). Quantification the expression levels of miR-22, NLPR3 and miR-22 targeting NLRP3 related proteins by means of qRT-PCR and Western blot. The colon cancer cell proliferation, invasion and migration were assessed via CCK8, colony formation and transwell assays. Subsequently, nude xenografts mice model was constructed and mice were divided into 4 groups: Model, miR-22, si-NLRP3 and miR + NLRP3. Through the changes of tumor volume and weight, the expression of Ki67 and the protein levels of NLRP3, IL-1B, MMP-9, MMP-2, E-catherine, N-catherine and Vimentin to verify the results.

Results

The cell proliferation, migration and invasion in miR-22, si-NLRP3 and miR + NLRP3 groups were significantly weaker than BC group. In compared to normal tissues, the growth rate of tumor was evidently slowed down, the volume and quality of tumor were evidently reduced and the proportion of Ki67 positive cells were evidently decreased. Both in tumor tissues and in cancer cells, the expression of IL-1B, NLRP3, MMP-9, MMP-2, N-Catherine and Vimentin were evidently decrease and the levels of E-Catherine were evidently increased than in normal tissues or cells. In compared to si-miR and CYLD groups, treatment with si-miR + si-C could effectively reverse the effect of suppressed the tumor progression.

Conclusion

miR-22 targets NLRP3 to inhibit the cell proliferation, migration and invasion in human colon cancer cell

Background

Colorectal cancer (CRC) is the third most ordinary cancer which prevails around all over the world, especially in developed world[1, 2]. The World Health Organization GLOBOCAN database [3] have revealed that there were more than over 1.8 million new cases every year. CRC is almost subsequently the
accumulation of the transformation of epithelial cells in the surface of the intestinal tract to cancerous cells[4, 5]. Rectal cancer, colon cancer and bowel cancer, are all the various types of CRC. More than half of the patients having suffering from the painful which from CRC or the surgery[6, 7]. The survival rate for CRC is less than 60% in recent years. The 5-fluorouracil therapy, tegafur plus uracil (UFT) against CRC has been developed as a more useful method in the past decade years. But, the varieties of CRC were successive reported in recent years. Therefore, there is an urgent need for in-depth understanding of the biological mechanisms in CRC in order to ask for more ways to therapy with CRC[8, 9]. In the past decennium, the cancer researches that based on the tumor-derived cell lines in human plays a cornerstone character in biology, which also have guided our understanding of the process of cancer development[10].

MicroRNA(miRNA) is a class of small non-coding RNAs which have a mean length of 21–25 nucleotides sequence[11]. Previous researches have demonstrated that miRNAs have an essential character in the post-transcriptional regulators of gene and non-coding RNA expression, thereby controlling the signaling events, cell migration, proliferation and basic various cellular pathways[12–14]. miRNAs also are function as a key element in tumor diagnosis and prognosis[15]. Therefore, miRNA expression profiles are related to tumor progression and are valuable in the clinical diagnosis and prognosis of most cancers[16]. Human chromosome 17p13.3 has a length of 22nt microRNA names miR-22, which was reported that the expressions were down-regulated in general cancer lines and function as a tumor suppressor. Once studies have reported that miR-22 frequently function as a cancer-regulator factor in several types of tumor including pancreatic cancer, breast cancer[17]. Current studies have revealed that miR-22 could interrupt the tumor progression including proliferation, migration and invasion to control the cancer symptoms in breast cancer and cervical cancer[17]. Moreover, the expression of miR-22 exhibits lower than normal tissues and through silence HIF-1α to disrupt the progression of CRC[18]. Increasing evidence have revealed that miR-22 function as a down-regulator by targeting BCL9L[19] and repressor by targeting RARb in the development of CRC[20]. Clinically, miR-22 displays the capability of treatment in several cancers.

In the present study, we performed the control and experiment group to reveal the function of miR-22 in human colon cancer cells, thereby revealed the potential mechanisms of miR-22 behind the repressed function in CRC. We also further verified that miR-22 inhibits the cell proliferation and invasion through targeting the expression of NLRP3.

**Methods**

**Cells culture**

Human colonic epithelial cell (HCoEpiC) and human colon cancer cell lines HCT116, HCT8, HT29, LS174T, LOVO, SW480 were all purchased from Shanghai Institute of Cell Research, CAS. These cells are grown in DEGM (GIBCO) mixed with 10% fetal bovine serum and 1% penicillin streptomycin in a 37 °C, 5%CO2 incubator. The cells in the logarithmic growth phase were selected to further study.
PT-PCR

The cells were collected and centrifuged at 4 °C (12,000 rpm) for 5 min. Total RNA was extracted using TRIzol reagent according to the manufacturer’s instruction (Tamara, Dalian, China). First-strand cDNA synthesis was performed by a cDNA Reverse Transcription Kit (Applied Biosystems, Waltham, MA, USA). The RT-PCR were performed by Mastercycles with 95 °C 15 s, 60 °C 60 s, 72 °C 40 s (35 cycles). Data were analyzed by the comparison Ct(2^{-\Delta\Delta Ct}) method and expressed as fold change relative to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) or U6.

The following primers were listed as follows:

- **miR-22 Forward**: 5’- GCATGGAAGCTGCCAGTTGAAG - 3’
  - **Reverse**: 5’- ATCCAGTGCGAGGTCCGAGG - 3’

- **U6 Forward**: 5’- CTCGCTTCGGCAGCACA - 3’
  - **Reverse**: 5’- AACGCTTCACGAATTTGCGT - 3’

- **NLRP3 Forward**: 5’- CCATCGGCAAGACCAAGA - 3’
  - **Reverse**: 5’- ACAGGCTCAGAATGCTCATC - 3’

- **GAPDH Forward**: 5’- TGACTTCAACAGCGACACCCA - 3’
  - **Reverse**: 5’- CACCCTGTTGCTGTAGGCCAAA - 3’

**Cell Transfection And Grouping**

Human HCT116 cells were prepared for following cell experiments. These cells were passaged for 24 hr and cultured in 6-well plates for lentiviral transfection [1]. The lentiviral particles were constructed by Shanghai Jikai Biotechnology Co., Ltd. The cells were randomly divided into 5 groups according to different methods:

1. Blank control (BC) group: no treatment.
2. miR-22 overexpression negative group (NC1) group: cells transfected with miR-22 scramble.
3. miR-22 overexpression group (miR-22): cells transfected with miR-22 mimic.
4. miR-22 silencing negative group (NC2): cell transfected with miR-22 inhibitor negative control.
5. miR-22 silencing group (si-miR): cell transfected with miR-22 inhibitor.
The expression levels of miR-22 and NLRP3 mRNA in cells after transfection 72 h was examined by RT-PCR.

**Cck-8 Cell Viability Assay**

Cancer cells were cultured in a 96-well plate at a density of $2 \times 10^4$ cells. Then, the culture medium was incubated for 4 h at 37 °C and followed with a time-series of concentrations of CCK-8 solution at 24 h, 48 h, 72 h, and 96 h. Finally, the optical density (OD) value at 450 nm was measured by a multifunction microplate reader.

**Colony Formation Assay**

Cells were detached with 0.25% trypsin and plated in a 6-well plate at 37 °C in a humidified incubator with 5% CO2 in air for 2 to 3 weeks, the fresh medium was changed every 3 days. The cells were fixed in methanol and each well was added with 1 mL Ji Giemsa working fluid and stained for 30 min. After washed with ultra pure water, the record was imaged by a camera.

**Transwell Assay**

Cell invasion and migration were all examined with the transwell assay. For invasion, pre-cooled DMEM medium was mixed with Matrigel (Solebao, Beijing) for a 1:1 dilution, then evenly spread in the upper chamber of the transwell (Corning Life Sciences, Corning, NY) at a density of 50 ul/well. Each well was added into 100 µl cell suspension, then incubated at 37 °C for 4 hr. Next, 600 µl of DMEM medium was added to the low chamber and cultured at 37 °C in a humidified incubator with 5%CO2 in air for 72 h. Then, the chambers was washed with PBS twice and fixed in 5% gluaraldehyde at 4 °C, followed by staining with 0.1% srystal violet. After 30 min, the Tanswell palate was placed under the inverted microscope (Olympus, Janpan) to observe the bottom of each chamber, from which five fields of view were selected randomly for cell counting. For migration, pre-cooled DMEM medium were not mixed with Matrigel and other steps were accordance with invasion experiment.

**Western Blot**

After the cells were lysed and centrifuged at 2000 rpm for 20 min, the supernatant was removed and used BCA kits (Solarbio, Beijing, China) to measure the protein concentration. Then, the cell suspension was gently mixed with 10% SDS-PAGE for a 1:1 dilution and heated at 95 °C for 5 min. Next, the proteins were transferred to PVDF membrane for 30 min and blocked with 5% bovine serum albumin (BSA) for 1 h. Followed by treating with the primary antibodies, including anti-MMP9 (ab73734, 1 µg/ml), anti-MMP2 (1:500, orb193343), anti-E-cadherin (1:500, orb43407), anti-N-cadherin (1:500, orb227888), anti-Vimentin (1:500, orb229187), anti-NLRP3 (1:500, orb319065), anti-all-1β (1:500, orb339111), anti-β-actin (1:2000,
and they were all incubated at 4°C overnight. All antibody are from Biorbyt, Cambridge, UK. After warming, these proteins were incubated with anti-rabbit IgG secondary antibody (1:1000, ABUN101998, antibodies-online, Aachen, Germany) for 1 h and washed with ECL for 3–5 min. Protein expressions were normalized by β-actin. Grayscale scanning and quantification were performed by Image J (NIH) software.

Luciferase Reporter Assay

The wild miR-22 sequence in the 3′-UTR of CYLD or a mutated variant were amplified in pGL3/Luciferase vector (Promega, Madison, WI, USA) and were cloned into the downstream of luciferase gene. A dual luciferase assay (Promega) was performed at 48 hours after transfection.

Verification

The cells were randomly divided into 6 groups: (1) Blank control group (BC), (2) miR-22 overexpression group (miR-22), (3) NLRP3 silencing negative group (NC3), (4) NLRP3 silencing group (si-NLRP3), (5) miR-22 overexpression + NLRP3 overexpression negative group (miR + NC4), (6) miR-22 overexpression + NLRP3 overexpression group (miR + NLRP3). RT-PCR was used to examine the expression of NLRP3 mRNA in cells after transfection for 72 h, and the above experiment was repeated.

Nude Mice Xenograft Models

Mice studies were implemented in 4 weeks old male BALB/c nude mice who weighted 16–18 g and were purchased from Beijing Vital River Laboratory Animal Technology Co., license number SCXK (Beijing) 20160006. All mice were fed in an independent cage with constant humidity at 24–26°C. Animal experiments were followed the NIH guidelines (NIH Pub. No. 85 – 23, revised 1996) and have been approved by the Animal Protection and Use Committee of Shengjing Hospital, China Medical University. Each logarithmic growth phase cell was digested with 0.25% trypsin and cell concentration was adjusted respectively to each group at 5 × 10^{-7} /mL [1]. The mice were injected intraperitoneally with 0.1 mL dilution on the dorsal skin of tight fore limb. Twenty-four nude mice were randomly divided into 4 groups: (1) model group, (2) miR-22 overexpression group (miR-22), (3) NLRP3 silence group (si-NLRP3) (4) miR-22 overexpression + NLRP3 overexpression group (miR + NLRP3). After the experiment, all animals were euthanized, the model animals were injected intraperitoneally with 0.6% sodium pentobarbital (50 mg/kg).

Tumor Volume Calculation

The long diameter (L) and short diameter (W) were counted to shape the tumor volume every 7 days. Tumor volume (V) = (long diameter x short diameter^2) / 2. After 28 days, the model animals were injected
intraperitoneally with 0.6% sodium pentobarbital (50 mg/kg), then weigh the tumor tissues. The tumor specimens were partially processed with 4% paraformaldehyde and embedded in paraffin for 24 h.

**Immunohistochemical Assay**

After routinely sectioning, the tumor specimens were dewaxed with xylene, and hydrated with a series ethanol solution. 3% H$_2$O$_2$ methanol solution was added to deactivated the samples for 20 min. Then add the citrate buffer (pH 6.0) to heat-fixed it in high temperature antigen for 10 min, and add 5% BSA to block it for 20 min. Polyclonal rabbit anti-human Ki67 antibody (1:200, orb88614, Biorbyt, Cambridge, UK) were added dropwise and reacted overnight at 4°C. After rewarming, the samples were incubated with goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:1000, ABIN101988, antibodies-online, Germany). The Slides were developed with DAB, then followed by counterstained, dehydrated, transparent, and sealed. The results were observed under a × 400 optical microscope (Olympus, Japan) and counted by Aperio Imagescope 11.1 software, and expressed as percentage (%) of positive cells.

**Statistical Analysis.**

Data were processed by SPSS19.0 software and presented as the mean ± SEM deviation. Statistical significance was conditioned by ANOVA for multiple comparisons and following analysis were performed by Turkey test. P < 0.05 was considered statistically significant.

**Results**

**Effect of miR-22 on cell proliferation, migration and invasion in human colon cancer cells**

The purpose of present study is aim at characterizing the functionality of miR-22 in human colon cancer cells. To elevate the ability of cell proliferation, migration and invasion, the expression levels of miR-22 in different groups were quantized. RT-qPCR results shown in Fig. 1 indicated that the mRNA levels of miR-22 were evidently decreased in human colon cancer cells, when compared to HCoEpic group (p < 0.05). The mRNA levels of miR-22 were up-regulated and the cell proliferation, invasion and migration were evidently down-regulated in the cancer cells of miR-22 group compared with BC group. In contrast, the mRNA levels of miR-22 were evidently down-regulated and the cell proliferation, invasion and migration were increased in the si-miR group compared with the BC group (p < 0.05). The cells in the si-miR group have a lower mRNA levels of miR-22 when compared with the miR-22 group. Moreover, the cell proliferation, invasion and migration were evidently increased in the si-miR group (p < 0.05). Taken together, these results confirmed the essential character of miR-22 in maintaining the regular cell proliferation, invasion and migration of human colon cancer cells.
Effect of miR-22 on the protein levels of MMP-9, MMP-2, N-cadherin, Vimentin and N-cadherin in human colon cancer cells

Consistent with previous study, the mRNA levels of miR-22 in human colon cancer cells prompted us to evaluate the expression levels of related proteins. Western blot analysis was performed to determine the protein levels of MMP-9, MMP-2, E-cadherin, N-cadherin and Vimentin in various cell groups (Fig. 2). The results show that in contrasted to the BC group, the protein expression of MMP-9, MMP-2, Vimentin and N-cadherin were poorly expressed, whereas E-cadherin was high expressed in the miR-22 group. The results were exhibited inversely in the si-miR group in contrasted with the BC group, the protein levels of MMP-9, MMP-2, Vimentin and N-cadherin were evidently increased and the protein levels of E-cadherin were evidently decreased (p < 0.05). Compared with the miR-22 group, the protein levels of MMP-9, MMP-2, N-cadherin and Vimentin were evidently increased, and the protein levels of E-cadherin were evidently decreased in the si-miR group (p < 0.05).

Effect of miR-22 on the expression levels of NLRP3 in human colon cancer cells

NLRP3 has been reported to be involved in the metastasis of many tumors. We also used western blot and RT-qPCR to detect the expression of NLRP3 in human colon cancer cells (Fig. 3A, B). TargetScan were performed to predict that NLRP3 is a target gene of miR-22 and miR-22 negatively regulates NLRP3, which confirmed by dual luciferase reporter assay (Fig. 3C, D). The results shown in Fig. 3 indicated that miR-22 inhibited the expression of NLRP3, whereas silencing the expression of mir-22 induced the expression of NLRP3 (p < 0.05). Compared with the BC group, the expression of NLRP3 and IL-1β protein was evidently decreased in the miR-22 group, while silencing miR-22 would reverse the downward trend (p < 0.05). Next, previous studies have determined the target relationship between miR-22 and NLRP3. In this study, we have developed a dual-luciferase reporter gene assay to verify the targeting effect of miR-22 on NLRP3. The results showed that the luciferase activity of miR-228 mimic in the recombinant plasmid NLRP3-Wt was found to be evidently decreased, whereas the luciferase activity in NLRP3-Mut showed no significant differences.

Effect of miR-22 targeting NLRP3 on proliferation, migration and invasion in human colon cancer cells

Next, to further confirm the results shown in the present study, we divided the cells into 6 groups again. Compared with the BC group, the expression of NLRP3 was evidently decreased, the cell proliferation, migration and invasion were also evidently decreased in miR-22, si-NLRP3, miR + NC4, and miR + NLRP3 groups (Fig. 4, p < 0.05). For miR-22 and si-NLRP3 groups, simultaneously treatment with miR-22 and
NLRP3 was evidently upregulated the expression of NLRP3 and the cell proliferation, migration and invasion (p < 0.05).

**Effect of miR-22 targeting NLRP3 on the expression of related proteins in human colon cancer cells**

To evaluate if miR-22 targeting NLRP3 could be a potential mechanism in human colon cancer cells, we examined the expression of related proteins using western blot. As shown in Fig. 5, the results indicated that the expression of NLRP3, IL-1β, MMP-9, MMP-2, Vimentin and N-cadherin proteins were evidently repressed in both miR-22, si-NLRP3, miR + NC4 and miR + NLRP3 groups. The effect was observed inversely in the expression of E-cadherin protein. These results were both when these groups compared with the BC group (p < 0.05). In addition, we observed an evidently enhanced expression of NLRP3, IL-1β, MMP-9, MMP-2, Vimentin and N-cadherin, associated to a evidently reduced of E-cadherin protein in the miR + NLRP3 group compared with both miR-22 and si-NLRP3 groups (p < 0.05).

**Effect of miR-22 targeting NLRP3 on the growth of human colon cancer xenografts**

To further confirm if miR-22 inhibits human colon tumorigenesis in vivo, we conducted a tumor formation experiment in nude mice to detect the tumor growth (Fig. 6). In comparison to the Model group, the tumor growth rate of mice in other groups was evidently slowed down, the tumor volume and quality were found to be evidently decreased, and the percentage of Ki67 positive cells in the tumor tissues was evidently decreased (p < 0.05). In compared to the miR-22 and si-NLRP3 group, the tumor growth rate of the miR + NLRP3 group was evidently accelerated, the tumor volume and quality were evidently increased and the percentage of Ki67 positive cells in the tumor tissue was evidently increased (p < 0.05).

**Effect of miR-22 targeting NLRP3 on the expression of related proteins in human colon cancer xenografts**

In compared to the Model group, the expression of NLRP3, IL-1β, MMP-9, MMP-2, Vimentin and N-cadherin protein was evidently decreased in the other groups, but the expression of E-cadherin protein was evidently increased (Fig. 7, p < 0.05). When In compared to miR-22 and si-NLRP3 group, the expression of NLRP3, IL-1β, MMP-9, MMP-2, Vimentin and N-cadherin were evidently increased and the expression of E-cadherin protein was evidently reduced in miR + NLRP3 group (p < 0.05).

**Discussion**
Previous reports have revealed that the potential functions of miRNA were discovered in various cancers including colon cancer, the changeable expression levels influenced many facets of tumor progression[21]. Up to now, the studies in excess of 20 have profiled the microRNA expression in CRC and verified the essential character in the process of cancer development[22]. Many microRNAs have differently expressed in tumor and nontumor cell lines and tissues, such as miR-143 and miR-145 were down-regulated in CRC, however, miR-21 were up-regulated in CRC[23]. Here, we examined the expression of miR-22 in cultured cell line and tumor tissue and found that the character of miR-22 was inclined to tumor inhibitor which consistent with other studies. We also discovered that the expression of miR-22 repressed CRC cell proliferation, migration and invasion, and these results were also supported in xenograft animal model. The underlying mechanisms of miR-22 regulated the tumor progression are as diverse and complex as other transcriptional factors. The more detailed parts of miR-22 in CRC is still worth dig up.

The immune system resists to other erosion via the expression of pattern recognition receptors (PRRs) which first reported in 2012 and the function of various inammasomes have been identified[24]. Nucleotide-binding domain and leucine-rich repeat-containing protein family (NLRs) as an important category which functioned in the underlying mechanisms of PRRS[25]. Previous reports have revealed that NLRP3 inammasome which formed by NLRP3 played an important character in activating the potently modulated innate immune function[26]. However, recent reports have varified that NLRP3 inammasome with excessive activation enhances the metastasis of various tumors including hepatocellular carcinoma cells and melanoma cells[27]. In this study, the results shown that NLRP3 was poorly expressed in the overexpression of miR-22 group and highly expressed in the silence of miR-22 group, which indicated that the expression of miR-22 is effectively repressed the expression of NLRP3. We also detected that the protein levels of IL-1β were consistent with the expression of NLRP3. To further investigated the effect of miR-22 targeting NLPR3, we also examined the expression of MMP-9, MMP-2, Vimentin, N-cadherin and E-cadherin. The different expression profile indicated that the potential function of these proteins in the progression of CRC.

Although the above results have demonstrated the functions of miR-22 targeting NLRP3 in human colon cancer cells. The underlying mechanisms of tumor tissues in vivo is remains unknown. To investigate these molecular puzzles, we performed the animal studies via constructing nude mice xenograft models. The results were also verified in tumor tissues. Consequently, we concluded that miR-22 targeting NLPR3 repressed the CRC cell proliferation, migration and invasion and plays the tumor suppressor character in tumor tissues.

**Conclusion**

In word, our study devotes to reveals that miR-22 function as a tumor suppressor in CRC and its underlying mechanism is majority relied on via targeting NLRP3. All results in the present study provide a fresh perspective to indicate that miR-22 is a potential prognostic biomarker and therapeutic target for CRC.
Abbreviations

HCoEpiC: Human colonic epithelial cells; CRC: Colorectal cancer; UFT: tegafur plus uracil; miRNA: MicroRNA; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; NLRP3: NLR family pyrin domain containing 3.

Declarations

All manuscripts must contain the following sections under the heading 'Declarations':

Ethics approval and consent to participate

Animal experiments were followed the NIH guidelines (NIH Pub. No. 85 – 23, revised 1996) and have been approved by the Animal Protection and Use Committee of Shengjing Hospital, China Medical University.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

Funding

Not applicable

Authors' contributions

JC and HZ carried out the experimental work and the data collection and interpretation. JG and JC participated in the design and coordination of experimental work, and acquisition of data. ZX and JC participated in the study design, data collection, analysis of data and preparation of the manuscript. JC carried out the study design, the analysis and interpretation of data and drafted the manuscript. All authors read and approved the final manuscript.
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Figures
Effect of miR-22 on cell proliferation, migration and invasion in human colon cancer cells. (A) The mRNA levels of miR-22 were examined by RT-qPCR in human colon cancer cells; (B) The mRNA levels of miR-22 were examined by RT-qPCR in transfected cells; (C) Cell proliferation was examined by CCK8; (D) Cell proliferation were examined by colony formation assay; (E) Cell migration were examined by the transwell assay (×400); (F) Cell invasion were examined by the transwell assay (×400). @p<0.05 vs. HCoEpiC group; *p<0.05 vs. BC group; #p<0.05 vs. miR-22 group. Data were processed by SPSS19.0 software and presented as the mean ± SEM deviation. Statistical significance was conditioned by ANOVA for multiple comparisons and following analysis were performed by Turkey test.
Effect of miR-22 on the levels of MMP-9, MMP-2, N-cadherin, Vimentin and E-cadherin proteins in human colon cancer cells. *p<0.05 vs. BC group; compared with #p<0.05 vs. miR-22 group. Data were processed by SPSS19.0 software and presented as the mean ± SEM deviation. Statistical significance was conditioned by ANOVA for multiple comparisons and following analysis were performed by Turkey test. The full-length are presented in material 1.

**Figure 3**

Effect of miR-22 on the expression of NLRP3 in human colon cancer cells (A) RT-PCR; (B) Western blot (C) Predict the binding site between miR-22 and the 3'-UTR regions of NLRP3. (D) Dual-luciferase reporter gene assay. *p<0.05 vs. BC group; #p<0.05 vs. miR-22 group; △p<0.05 vs. miR-NC group. Data were processed by SPSS19.0 software and presented as the mean ± SEM deviation. Statistical significance was conditioned by ANOVA for multiple comparisons and following analysis were performed by Turkey test. The full-length are presented in material 2.
Figure 4

Effect of miR-22 targeting NLRP3 on proliferation, migration and invasion in human colon cancer cells. (A) RT-PCR; (B) CCK8; (C) Colony Formation Assay; (D) Transwell (cell invasion) (×400); (E) Transwell (cell migration) (×400). &p<0.05 vs. model group; #p<0.05 vs. miR-22 group; ^p<0.05 vs si-NLRP3 group. Data were processed by SPSS19.0 software and presented as the mean ± SEM deviation. Statistical significance was conditioned by ANOVA for multiple comparisons and following analysis were performed by Turkey test.

Figure 5

Effect of miR-22 targeting NLRP3 on the expression of related proteins in human colon cancer cells. &p<0.05 vs. model group; #p<0.05 vs. miR-22 group; ^p<0.05 vs si-NLRP3 group. Data were processed by SPSS19.0 software and presented as the mean ± SEM deviation. Statistical significance was conditioned by ANOVA for multiple comparisons and following analysis were performed by Turkey test. The full-length are presented in material 3.
Figure 6

Effect of miR-22 targeting NLRP3 on the growth of human colon cancer xenografts. (A) tumor volume; (B) tumor photograph; (C) tumor weight; (D) immunohistochemistry to detect Ki67 expression in xenografts (×400). &p<0.05 vs. model group; #p<0.05 vs. miR-22 group; ^p<0.05 vs si-NLRP3 group. Data were processed by SPSS19.0 software and presented as the mean ± SEM deviation. Statistical significance was conditioned by ANOVA for multiple comparisons and following analysis were performed by Turkey test.
Figure 7

Effect of miR-22 targeting NLRP3 on the expression of related proteins in human colon cancer xenografts. &p<0.05 vs. model group; #p<0.05 vs. miR-22 group; ^p<0.05 vs si-NLRP3 group. Data were processed by SPSS19.0 software and presented as the mean ± SEM deviation. Statistical significance was conditioned by ANOVA for multiple comparisons and following analysis were performed by Turkey test. The full-length are presented in material 4.

Supplementary Files

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