Effect and mechanism of miRNA to osteosarcoma cell

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Abstract: MicroRNA has proved to be low expression in many tumor cells. In addition, it was also proved that as a kind of cancer suppressor gene, miR-199a-3p in miRNA can affect the growth and invasion ability of tumor cells. This paper aims to discuss the effect of miRNA to osteosarcoma cell. It used synthetic mature miR-199a-3p sequence simulants to transfect osteosarcoma cell and took negative contrast sequence (NC mimics) transfection cell as negative contrast. After transfection, qRT-PCR was applied to detect the expression quantity of miR-199a-3p in every group. Western blot method was applied to detect the expression level of MCL-1 protein and shear situation of PARP in groups of cells. Flow cytometry was used for detecting apoptosis rate of cells and the experimental result was made a statistical analysis. The result shows that in cells from experimental group of transfection miR-199a-3p sequence simulants, expression quantity of mi-R-199a-3p significantly increased while MCL-1 protein expression decreased compared to control group. In addition, shear level of PARP protein and apoptosis rate of cells increased. The differences all had statistical significance ($P<0.05$). It was concluded that miR-199a-3p can effectively promote the apoptosis rate of osteosarcoma cells.

Keywords: miR-199a-3p, osteosarcoma, effect.

INTRODUCTOIN

Osteosarcoma is the second most common malignant bone tumor after myeloma. It stems from bone mesenchymal cells and is always an intractable disease intruding human health (Zhao *et al.*, 2013). People would be tremble with fear when mention it. It is because that it brings serious torment to us and also because the period from early stage to late stage of it is short. Once it is found, family would suffer destructive hit. miRNA is a kind of non-coding micromolecule found in recent years. It has length of 22 nucleotides and is widely existed in eucaryon. miRNA participates in generation and development of various tumor, which is closely related to the generation, development, invasion and metastasis of osteosarcoma. It plays the function of oncogene and cancer suppressor gene. Therefore, miRNA is thought to have the potential of becoming new effective tumor marker and therapeutic target (Duan *et al.*, 2011). Research found that miR-199a-3p showed low expression in tumor such as hepatocellular carcinoma (Fornari *et al*, 2010), osteosarcoma (Duan *et al.*, 2011), carcinoma of urinary bladder (Ichimi *et al.*, 2009), ovarian cancer (Iorio *et al.*, 2007) etc., which was closely related to the invasion and metastasis of cells. However, there are fewer reports on the expression and effect of miR-199a-3p in osteosarcoma. Osteosarcoma cells were transfected and the expression of miR-199a-3p in osteosarcoma cells was up-regulated through the synthesis of miR-199a-3p stimulant (Fan *et al.*, 2000; Cheng and Yang, 2013, Asghar *et al.*, 2003; Jaffe, 2010). The expression change of MCL-1, shear situation of PARP and the change of cells apoptosis

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METHODS

Cultivation and transfection of cells

In the condition of 37°C and CO_2 with 5% volume fraction, osteosarcoma cells were put into DMEM medium containing 10% fetal calf serum for cultivation. Nutrient solution was changed every 36 h. Logarithmic phase cells were inoculated in 6-hole board. The experiment was divided into three groups: blank control group (no processing), negative control group (transfection NC mimics) and experimental group (transfection miR-199a-3p mimics). When area of osteosarcoma cells was up to 75%, then medium should be changed. Cells were gained for latter testing 8h after transfection of reagent LipofectamineTM2000 and OPTI-MEM transfecting simulant and 48h after solution change.

Detection of expression quantity of Mir-199a-3p

First, TRlzol kit was used to extract total RNA in groups of cells and spectrophotometer was used for quantitatively detecting sample concentration and whether RNA is qualified. Qualified RNA sample can use TaKaRa RT-kit and fluorescent qPCR kit to detect different expression of miR-199a-3p in three samples. In the condition of 37°C for 15 min and 85°C for 10 s, RT reaction started. U6 was selected for internal reference qRT-PCR reaction. The reaction procedure is: segment 1 (cycle 1; 90°C 30s), segment 2 (cycle 40; 90°C 15s; 60°C 30s), segment 3 (cycle 1; 90°C 15s; 60°C 54s;

90□15 s). Ct values of internal reference and target gene in every sample were gained after reaction. Expression quantity of miR-199a-3p in blank control group was expressed as 1. According to formula 2-△△CT

[▵▵Ct=(Cttarget-CtU6)test-(Cttarget-CtU6) calibration], relative content of miR-199a-3p in negative group and experimental group can be derived. Then according to three experimental results, average value and standard derivation of gene relative expression quantity in groups can be derived. Then these data were used for fig. drawing and done statistical analysis.

Detection of MCL-1 protein expression quantity and PARP shear situation

First, SDS-PAGE was used to electrophoretically separate protein in groups of cells. Then the protein was transferred to NC mimics. TBST solution (Tris Buffered Saline, with Tween-20, pH8.0) was added into skim milk powder to prepare 50 ml confining liquid. It was stored in 5□ refrigerator overnight. Confining liquid was poured out and washed the mimic for three times by TBST solution in 10 min every time. Then TBST solution was poured out and added anti-MCL-1 (1:1000), anti-β-actin (1:3000) and anti-PARP (1:1000). It was put in shaking table in indoor temperature for 2 h of slow incubation. Primary antibody was absorbed and washed mimics for three times by TBST 10 min for every time. TBST was poured out and respectively added relative second antibody. It was put in shaking table for 40 min of slow incubation. Second antibody was absorbed and washed mimics for three times 10 min for every time. TBST was poured out and added moderate developing substrate. We observed concentration change of MCL-1 protein bands and shear situation of PARP. Then Image-J software was applied to detect β-actin protein and gray level ratio of MCL-1 protein bands and make semi-quantitative on expression of MCL-1 protein.

Detection of apoptosis rate of cells after transfection

Cells were collected 48 h after transfection for preparation of single-cell suspension. It was washed for two times by PBS (phosphate buffered solution). Annexin V-PI was used for double dye according to operational instruction of apoptosis detection kit. Then it was placed keeping out of the sun for 10 min. At last, flow cytometry was applied to detect the apoptosis rate of cells.

RESULTS

Relative expression quantity of Mir-199a-3p in cells after transfection

qRT-PCR technology was applied to detect relative expression quantity of miR-199a-3p in experimental group and negative control group. The experiment was repeated for three times The detection result showed that experimental group was (8.24±0.55), negative group was (1.51±0.50) and expression quantity of blank control group was supposed as 1; expression of miR-199a-3p in cells of experimental group significantly increased. Compared to control group, its difference had significant statistical meaning ($P<0.01$, as shown in fig. 1).

MCL-1 protein content and PARP shear situation in cells after transfection

Groups of cells were collected after 48 h of transfection. Expression quantity of MCL-1 protein and shear situation of PARP protein were detected with β-actin as internal reference. The experiment was repeated for three times. Expression effect of protein was that experimental group (5.79±0.50), negative control group (2.04±0.19) and blank control group (1.87±0.15). MCL-1 protein expression quantity of cells in experimental group significantly decreased. Compared to control group, difference had statistical meaning ($P<0.01$, as shown in fig. 2). Compared to control group, overall fragment of PARP protein in experimental group decrease. Shear situation was significantly higher than that of control group.

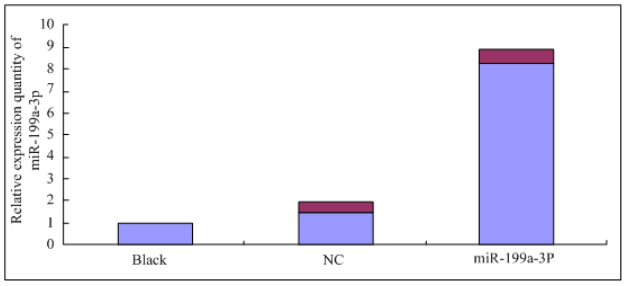

Fig. 1: Relative expression quantity of miR-199a-3p in cells after transfection

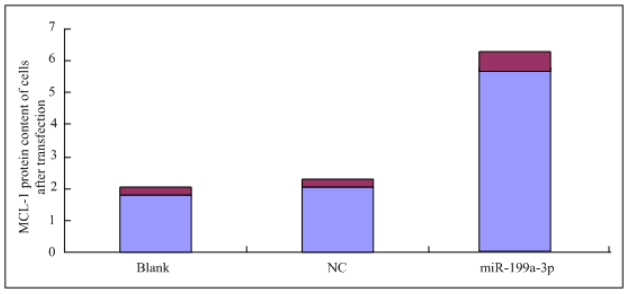

Fig. 2: MCL-1 protein content of cells after transfection

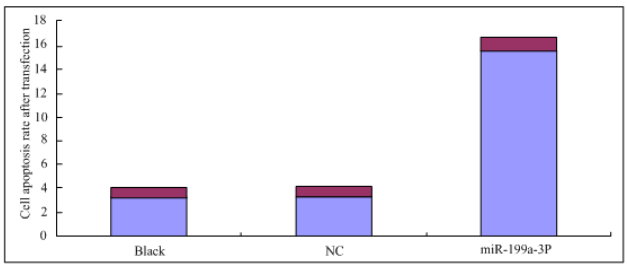

Fig. 3: Cell apoptosis situation after transfection

Cell apoptosis after transfection

Apoptosis rate of cells were detected. The experiment was repeated for three times. Apoptosis rate of experimental group is (15.33±1.42) %. Negative control group is (3.43±0.80)%. Blank control group is (3.35±0.70) %. Cell apoptosis rate of experimental group significantly

increased. Compared to control group, difference had statistical meaning ($P<0.01$, as shown in fig. 3).

DISCUSSION

Osteo sarcoma is a kind of malignant connective tissue tumor. It has high malignant degree and weak prognosis. Morbidity of it takes the first place in all primary malignant tumor. Threat brought by osteosarcoma is serious and needed to be attached importance. Different therapeutic method was applied according to different malignant degree. There are many kinds of malignant tumor such as tumor with high malignant speed and high transfer rate and tumor with low malignant speed and low transfer rate. Complete resection can be applied in tumor with low malignant degree. In most cases, it can be cured. However, facing with these tumor with high malignant degree, 5 years survival rate will not exceed 70% even when operation combined with chemotherapy regimens are applied,. Under the circumstance of non-ideal therapeutic effect of osteosarcoma, the critical point is using gene level to cure osteosarcoma and finding effective therapeutic target (Hellwinkel *et al.,* 2005; Wu *et al.*, 2011; Zhu *et al.*, 2012; Kang *et al.,* 2012). Research showed that miRNA can adjust many kinds of important biological process. miRNA plays a leading role in adjustment of signal channel, differentiation, apoptosis and proliferation of cells and tumor progress. It can be used as effective cancer suppression gene. Research proved that for 70% of B-CLL patients, expression of miR-15 and miR-16 significantly decreased and are even deficient. miR-15 and miR-16 mainly inhibit tumor by decreasing expression of anti-apoptosis protein Bcl-2. miRNA is not only cancer suppressor gene but also cancer gene. Research illustrated that miR-155can play a strong carcinogenic effect by mutual function with cancer gene mycm. miR-372 and miR-373 can activate cyclin dependent kinase 2 (CDK2) to inhibit activity of cancer suppressor gene and promote the growth of tumor. miRNA can also regarded as cancer suppressor gene and cancer gene in the same time. For instance, inhibition of miR-24 expression in Hela cell can promote cell proliferation. However, decrease of miR-24 expression in alveolar cell carcinoma can significantly inhibit cell proliferation.

In recent years, many researches found that many kinds of miRNA play a critical function on production and development of osteosarcoma. They play an important role when participating in many biological process such as invasion, transfer and drug resistance of osteosarcoma (Song *et al.*, 2009; Kobayashi *et al.*, 2012; Fan *et al.*, 2012). miR-199a-3p is a kind of miRNA that have tumor inhibition function. It had been proved that it shows low expression in many kinds of tumor cell. Research of Duan *et al* shows that miR-199a-3p shows low expression in osteosarcoma tissue and it inhibits invasion and transfer of osteosarcoma cell by decreasing expression of mTOR./Met and Stat3. This paper made further research on the effect and mechanism of miR-199a-3p in cell apoptosis of osteosarcoma. Initial research of effect of miR-199a-3p in osteosarcoma cell was carried out by *in vitro* cultivation osteosarcoma cell, transfecting cells by miR-199a-3p sequence stimulant and obtaining osteosarcoma cell with high content of miR-199a-3p. The experiment shows that content of MCL-1 in experimental group with high expression miR-199a-3p significant decreased compared to control group (Fan *et al.*, 2012). In addition, PARP is cutting substrates of core member of cell apoptosis caspase. Activation of PARP is the important mark of cell apoptosis. Overall fragment of PARP in experimental group decreased. Spliceo some content increased and pyrolysis activation appeared. It illustrated that cell apoptosis of experimental group was more significant than that of control group. Detection of apoptosis rate by flow cytometry found that cell apoptosis of experimental group with high expression miR-199a-3p was significant higher than that of control group. The difference had statistical meaning.

miRNA has important potential in promoting diagnosis and treatment of osteosarcoma. As a new emerging and effective gene therapeutic tool, miRNA has broad application prospect in new anti-cancer medicine development and treatment. This research illustrates that miR-199a-3p can significantly promote osteosarcoma cell apoptosis. Expression quantity may be used as a new important index for judging prognosis. miR-199a-3p may be become a new gene target for osteosarcoma treatment with the constant development of research. In addition, mechanism of miR-199a-3p to osteosarcoma cell apoptosis waits for further study because the research was lack of *in vivo* test proof. Moreover, there are still some drawbacks for practical application of miRNA in diagnosis and treatment of osteosarcoma such as bad stability and bad targeting of artificial synthesis miRNA (Jin *et al.*, 2011; Wu *et al.*, 2011), which hinder its clinical application. Once critical problem of miRNA technology development is solved, iatrotechnics reformation would start. Therefore, research and application of miRNA technology is of great significance.

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