



## The influence of MDR1 expression regulated by miR-138 through TRPS1 signaling pathway on multidrug resistance of osteosarcoma and formation of bacterial infection biofilm

Xiang Fang<sup>1#</sup>, Lizhen Zhu<sup>2#</sup>, Qiang Wu<sup>3</sup>, Xueren Zhong<sup>3</sup>, Yu Chen<sup>3</sup>, Wengang Zhu<sup>3\*</sup>

<sup>1</sup>The Medical College, Shantou University, Shantou, 515041, Guangdong province, China

<sup>2</sup>The Medical Record Room, Yuebei People's Hospital Affiliated to Shantou University Medical College, Shaoguang, 512025, Guangdong Province, China

<sup>3</sup>Department of Joint Orthopedics, Yuebei People's Hospital Affiliated to Shantou University Medical College, Shaoguang, 512025, Guangdong Province, China

#These authors contributed equally to this work as co-first author

### ARTICLE INFO

#### Original paper

#### Article history:

Received: February 03, 2023

Accepted: April 18, 2023

Published: May 31, 2023

#### Keywords:

Biofilm, micro ribonucleic acid, multidrug resistance gene 1, osteosarcoma, trico-nasal finger syndrome 1

### ABSTRACT

This study was to investigate the effect of microribonucleic acid (mi-RNA) on the resistance of human multi-drug resistance gene 1 (MDR1) to osteosarcoma through the Trico-nasal finger syndrome 1 (TRPS1) pathway, as well as the effect of mi-RNA on biofilm formation. For this purpose, firstly, the expression of MDR1 and TRPS1 in osteosarcoma cells was detected by quantitative polymerase chain reaction (qPCR) technology. Moreover, the clinical paraffin sections of osteosarcoma cells were collected to explore the correlation between MDR1 and TRPS1. Then, both the MG-38 cells expressing and not expressing miR-138 were expanded. Afterward, a plasmid with a full-length clone of the TRPS1 antibody was applied to transfect the cells. Besides, Q-OCR was employed to detect the expression of TRPS1 and MDR1, and the expression of TRPS1 protein and P-glycoprotein (P-gp) was detected by Western blot (WB). The MTT method was adopted to detect the changes in the median lethal dose of doxorubicin and cisplatin in cells from each group. The well plate was used to establish an in vitro bacterial infection biofilm model, and the above two transfected cells were added during the model establishment process. Moreover, the formation of biofilm in the two groups was observed. The result of the paraffin biopsy was 33% (25/75) of mi-RNA, the positive rate of TRPS1 was 18.6%, and the Pearson correlation coefficient of the two was 0.477. Under mi-RNA interference, the TRPS1 and MDR1 of the three system cells were sharply reduced, and the trend of changes between the two was the same. The tolerance of the mi-RNA interference group to doxorubicin, cisplatin, paclitaxel and 5-fluorouracil decreased steeply, and the median lethal dose dropped, while the non-mi-RNA interference group showed the opposite trend. In addition, the number of colonies in the interference group was less sharp than that of the control group and the non-mi-RNA interference group. The conclusion was that mi-RNA could control the expression of MDR1 through the TRPS1 pathway, thus affecting the multi-drug resistance of osteosarcoma and also influencing the formation of bacterial biofilms.

Doi: <http://dx.doi.org/10.14715/cmb/2023.69.5.9>

Copyright: © 2023 by the C.M.B. Association. All rights reserved.

### Introduction

Osteosarcoma is a very common primary malignant bone tumor with a high incidence, ranking second among many primary malignant tumors (1). This tumor mostly originated from mesenchymal tissue, with a very high degree of malignancy. Besides, its occurrence location is mostly located in soft tissue and local bone, so its destructive power and invasion are very high, and it is easy to be metastasized in the early stage. In addition, the majority of patients are children and adolescents, so the prognosis is generally poor (2). It can appear as lung or distant tumor metastasis in just a few months. At the current medical level, the 5-year survival of patients is still not very good even if early detection and early treatment are achieved by careful classification before surgery and standard treat-

ment after surgery (3).

Currently, the treatment of bone tumors mainly relies on chemotherapy drugs and neo-adjuvant chemotherapy, and these methods have relatively ideal therapeutic effects on bone tumors (4). With the more and more extensive use of chemotherapy therapy in clinical practice, it has been found recently that different patients have different sensitivities to various types of chemotherapy drugs in the treatment process, and some patients have shown increasingly poor sensitivity to the use of chemotherapy drugs. The severity of these phenomena affects the treatment effect and the adverse outcome of the patients. The phenomenon of tumor cells' sensitivity to chemotherapy drugs is called drug resistance, which is usually divided into primary drug resistance (PDR) or natural drug resistance and secondary or acquired drug resistance (5). The first type is at the be-

\* Corresponding author. Email: [qiaoguan421962405@163.com](mailto:qiaoguan421962405@163.com)

ginning of the use of chemotherapeutic drugs, which have poor or no effect on tumor cells. The second type is that drugs have good effects on tumor cells in the early stage of use, but the effect of chemotherapy drugs is not as good as the original effect as the treatment progresses. According to the different ranges of targeted drugs, it can be divided into PDR and MDR. The so-called active drug resistance refers to the resistance to the applied drug (6). MDR refers to resistance caused by one drug, and resistance to not only the initiator but also to other drugs with different structures and mechanisms of action, also known as cross-resistance. Studies have shown that the high expression of P-gp is the main cause of drug resistance in tumor cells (7). P-gp can bind to chemotherapeutic drugs through specific sites, and pump drugs out of the cell against the concentration gradient, resulting in a decrease in intracellular drug concentration, to affect the therapeutic effect of drugs. Studies have confirmed that reducing the expression of P-gp can effectively improve the sensitivity of tumor cells to chemotherapy drugs. Therefore, reducing P-gp can be used as an idea to improve the drug sensitivity of tumor cells (8).

Micro ribonucleic acid (mi-RNA), a non-coding RNA, is also an endogenous regulator, which has effects on the degradation and translation of messenger RNA (mRNA) and can also be counter-complementary to mRNA. Studies have screened mi-RNA in tumor cells of patients with osteosarcoma, finding that the expression of miR-138 has been significantly increased. What's more, it has also indicated that mi-RNA also has a greater effect on TRPS1. Moreover, TRPS1 is a gene that can mediate TRPS, which has a section of transcriptional regulators containing 9 zinc proteins. Studies have shown that it has a certain guiding significance in breast cancer and prostate cancer. Recent studies have found that it has a certain correlation with human multidrug resistance gene 1 (MDR1). Furthermore, MDR1 can encode a pump protein P-gp that can be embedded in the cell membrane, and the influence of this protein on the drug resistance of tumor cells is crucial. Once the expression of this protein enhances the exclusivity of chemotherapeutic drugs in tumor cells, which leads to tumor cells showing resistance to chemotherapeutic drugs, especially doxorubicin and paclitaxel. Therefore, it can be inferred that mi-RNA plays a certain role in improving the MDR of osteosarcoma (9).

A biofilm is defined as a membrane-like structure made up of a variety of bacteria that are attached to damaged tissue or solid surfaces for their growth and development. Biofilm can protect bacteria and antigens, making it difficult for drugs to have a substantial effect on bacteria, so it is easy to lead to the emergence of refractory infections once the biofilm forms. The biofilm not only has a strong resistance to host immunity and is difficult to be removed, but also stimulates the host to produce high-valence antibodies, form immune complexes, and cause local tissue damage. Osteosarcoma is a kind of malignant tumor in the course of its treatment, it is inevitable to carry out some invasive examinations. These invasive examinations

have resulted in an increasing number of biofilm-associated infections during the treatment of osteosarcoma. Based on the above background, it is necessary to investigate the formation of biofilm. The research data have shown that the main components of the biofilm are extracellular polysaccharide (PLA), an extracellular protein, and extracellular deoxyribonucleic acid (DNA). Studies have shown that mi-RNA has a certain influence on the transcription factor Rbf that determines the formation of extracellular PLA and extracellular protein (10). It is suggested that the control of biofilm formation by controlling mi-RNA may serve as a way to avoid intractable infections during the treatment of osteoma. In this study, the influence mechanism of mi-RNA on MDR1 in osteoma through the TRPS1 signaling pathway and its influence on the formation of biofilm were explored to provide a reference and basis for the prevention and treatment of related diseases in clinical practice. Based on the above background, MDR1 and TRPS1 were taken as the entry point in this study to explore the influence and mechanism of miR-138 on the resistance of osteosarcoma cells and its influence on the formation of bacterial biofilm, thereby providing new ideas and reference for the treatment of clinically related diseases.

## Materials and Methods

### Research objects

All human osteosarcoma-sensitive cell lines were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences. U2-OS (a widely used osteoblastic cell line derived from osteosarcoma) and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) cells were cultured on high glucose Dulbecco's modified eagle medium (DMEM), and Saos-2 cells (human osteoblastic cells) were cultured on McCoy's 5A medium. The specific information of each classical cell line was shown in Table 1. The plasmid vector was pSUPER.neo+GFP, and the eukaryotic vector was pcDNA3.1(+). Staphylococcus aureus was purchased from Ningbo Mingzhou Biotechnology Co., Ltd. Methoxylin-resistant Staphylococcus aureus (MRSA) was isolated clinically. Clinical gross samples of 75 patients with osteosarcoma collected, who did not receive neoadjuvant chemotherapy in Hospital X. The tissue sections were fixed with formalin and embedded with paraffin. All relevant tissue tests were completed by the Pathology Department of X Hospital. The experiment done in this study obtained the informed consent forms from patients and met the requirements of medical ethics (the license number should be provided).

### Chemical staining of tissues

The tissue sections were dewaxed and hydrated using routine methods, and then, washed with phosphate buffer saline (PBS) for 5 minutes each time (a total of 3 times). Then, the sections were incubated in a 3% hydrogen peroxide solution prepared with PBS phosphate buffer for 10-15 minutes. Besides, they were rinsed 3 times with

**Table 1.** Information of classical cell lines of each system.

Cell lines	Sources	Culture dish
U2-OS	Shanghai Cell Bank of the Chinese Academy of Sciences	DMEM
Saos-2	Shanghai Cell Bank of the Chinese Academy of Sciences	McCoy'S 5A
MNNG/ HOS C1#5	Shanghai Cell Bank of the Chinese Academy of Sciences	DMEM

PBS buffer for 5 minutes each time. Next, the 0.01M of sodium citrate buffer solution was microwaved to 95° and added with the tissue sections to heat at a constant temperature for 15 minutes, which was naturally cooled to room temperature. Then, PBS buffer was employed to rinse 3 times, with 5 minutes each time. TRPS1 and P-gp primary antibody working solution were dropped into the above, which were incubated overnight at 4°C. It was rinsed 3 times again, with 5 minutes each time. One drop of DAB Plus Chromogen was added into 1 mL of DAB Plus Substrate, which was mixed well and added to the sections. Afterward, they were incubated for 2 minutes to observe the staining conditions under a microscope. Finally, the sections were rinsed in clear water, and then, stained with hematoxylin. After the sections were rinsed again with clear water, hydrochloric acid and alcohol were added for differentiation. The sections were returned to blue with ammonia water and rinsed properly until they were transparent and eventually sealed.

### Extraction of cellular RNA

The culture solution was aspirated from each well of the tumor cell culture well. Then, both sides were washed with PBS and added 0.5 mL of Trizol. Next, the adherent cells were blown down through a micropipette, mixed well, and placed at room temperature. After the cells were lysed for 5 minutes, the lysate was aspirated and placed in a pure PE tube that was free of RNA and pollution. 1/5 Trizol reagent volume of chloroform reagent was added into each EP tube, and then, each EP was placed on a high-frequency oscillator and vigorously oscillated for 30 seconds. They were left for 5 minutes and centrifuged at 12,000 rpm for 5 minutes at 4°C. Then, the upper layer of the EP tube was moved to a new EP tube pretreated with DEPC water, and an equal amount of isopropanol was added to the tube. It was mixed well and left at room temperature for 10 minutes. After centrifugation at 12,000 rpm for 10 minutes at 4°C, the supernatant was removed. 0.5 mL of 75% ethanol solution prepared with DEPC water was added into the EP tube, mixed well, placed on a high-frequency shaker, and vortexed vigorously. Moreover, it was centrifuged at 7,500 rpm at 4°C for 5 minutes. The supernatant was removed, and the precipitate was saved and dried. What's more, the precipitate was dissolved with 10-20 uL of RNA dissolving solution. Afterward, the concentration and absorbance of the obtained RNA were measured by the nucleic acid-protein analyzer.

### Reverse transcription experiment and real-time fluorescence qPCR

**Reverse transcription experiment:** The operation strictly followed the corresponding operating procedures and instructions of the reverse transcription reagent. The reaction system was equipped based on Table 2. The specific operation steps were as follows: 37°C reverse transcription for 15 minutes, and 98°C enzyme-inactivation for 5 minutes. After the reaction, the concentration and purity of the obtained complementary DNA (cDNA) were measured by a protein quantifier and stored at -20°C or -80°C for later use.

**Real-time fluorescence qPCR:** Follow the steps on the kit strictly for real-time fluorescence qPCR detection. The specific reaction system and operation steps were as follows.

Reaction conditions included the following. Reaction steps: pre-denaturation at 95°C for 10 minutes, denaturation at 95°C for 15 seconds, and annealing/extension at 60°C for 1 minute, with a total of 40 cycles. Dissolution curve analysis: 95°C for 15 seconds, 60°C for 1 minute, 95°C for 15 seconds, and 60°C for 15 seconds.

### Plasmid transfection of osteosarcoma cells

The plates were inoculated with osteosarcoma cell lines before transfection, and then, the plates were placed in an incubator for 12-14 hours until the cell fusion was 70%-90%. Then, 400 uL of serum-free high glucose DMEM medium, 4 µg of plasmid DNA, and 6 uL of transfection agent were mixed in an EP tube and incubated at 37°C for 15 minutes. Afterward, the culture broth on the cell culture plate was removed, and the plate was washed twice with PBS. 3.6 mL of fresh culture solution was added, and 400 uL of transfection mixture was slowly dropped into the culture and mixed for further culture. Relevant experiments could be carried out 48-72 hours after transfection.

### Dosing MTT test

After the digestion, the counted cells of each group were inoculated on a 96-well culture plate with  $8 \times 10^3$  cells per well. After the cells were fully attached, different concentrations of chemotherapeutic drugs were added to each group of cells. Besides, the concentration gradient of doxorubicin was 0, 0.04, 0.2, 1, and 5 ng/mL; the concentration gradient of adriamycin was 0, 0.04, 0.2, 1, and 5 ng/mL; the concentration gradient of cisplatin was 0, 0.04, 0.2, 1, and 5 ng/mL; the concentration gradient of paclitaxel was 0, 0.04, 0.2, 1, and 5 ng/mL; the concentration gradients of 5-fluorouracil were 0, 0.0625, 0.25, 1, and 4 µg/mL. In addition, there were blank control wells containing culture medium, 4 replicate wells for each concentration, and the liquid volume of each well was 100 uL. Then, the orifice plate was put in an incubator for 48-72 hours. Afterward, the culture solution was sucked up, and 20 uL of fresh medium containing 5mg/mL MTT solution was added into each well, and then, they were continuously incubated for 2 hours. After the culture medium was removed, 100 uL of dimethylsulfoxide (DMSO) was added to

**Table 2.** Reverse transcription reaction system.

RNA	1 ug
5×RT Buffer	2 uL
RT Enzyme Mix	0.5 uL
RT Enzyme Mix	0.5 uL
Primer Mix	0.5 uL
Primer Mix	0.5 uL
Nuclease-free Water	Supplement to 10 uL
Total volume	10 uL

**Table 3.** qPCR reaction system.

Reagents	Volume	Final concentration
2×UltraSYBR Mixture(With ROX)	9.5 uL	1×
Forward Primer, 10 uM	0.5 uL	0.25uM
Reverse Primer, 10 gM	0.5 uL	0.25uM
Template cDNA	1 uL	
RNase-Free Wwater	8.5 uL	



each well and the plate was shaken on the shaker for 10 minutes. Finally, the absorbance at 570 nm was measured with a microplate analyzer. Furthermore, the experiment was repeated three times (11).

### Establishment of the bacterial biological model in vitro

A fresh single colony was picked out from the check-out ring and placed in 5 mL of tryptone soya broth (TSB) for incubation at 37°C for 24 hours. Then, TSB broth was applied to adjust the concentration of the broth to about 106 cfu/mL. The broth was added into two 96-well plates, with 200  $\mu$ L each. Moreover, the cells expressing mi-RNA and those not expressing mi-RNA were added to the two-pore plates. Afterward, the orifice plate was covered and incubated in a 37°C incubator for 72 hours. The orifice plate was removed and rinsed with normal saline. 25  $\mu$ L of 1% crystal violet solution was added to each well and dyed for 20 minutes. After the orifice plates were rinsed with normal saline again, 200  $\mu$ L of 95% alcohol was added to each well, which was left for 5 minutes. Finally, the optical density (OD) value of the wavelength at 580 nm was measured by a spectrophotometer (12).

### Western blot (WB) experiment

The pre-prepared and solidified sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) glue was installed in the vertical electrophoresis device, a sufficient amount of electrophoresis buffer was added to the electrophoresis tank, the comb on the gel was pulled out horizontally, the sample holes were rinsed, and the residual gel was washed out. Afterward, the electrophoresis tank was connected to the electrophoresis apparatus for pre-electrophoresis, and the voltage was kept at 60V for 10 minutes. 20  $\mu$ g of protein samples were added to each sample adding well in sequence, 3  $\mu$ g of protein marker was added to the reserved protein marker well, 20  $\mu$ g of loading buffer was added to the non-sample well, and constant pressure 60V electrophoresis was conducted. 40 minutes later, the constant voltage was increased to 100V, and the electrophoresis was continued until the separation of the protein marker was visible and bromophenol blue was close to the bottom of the gel. Then, the gel was carefully removed and placed in the wet transfer membrane buffer. According to the bands of pre-dyed protein Marker separated by visual separation and the molecular weight of target protein and internal reference protein, the gel containing the corresponding protein was cut off, the appropriate size thick filter paper and poly (vinylidene fluoride) (PVDF) membrane were cut out, and the PVDF membrane was put in methanol for activation for 3 minutes. The wet electrophoresis solution was placed with the thick filter paper-PVDF membrane-glue-thick filter paper in order, to drive out the bubbles in the middle. The end of the PVDF membrane was close to the anode. The above "sandwich" was placed in a wet electrophoresis tank and added with a sufficient electrophoresis solution and ice bag. Next, the electrode was connected, the constant pressure was 100V, and the membrane was transferred for 1 hour. After the membrane transfer, the PVDF membrane was taken out, and at this time clear protein Marker bands should be visible on the membrane. 5% skimmed milk powder solution was prepared with tris buffered saline (TBS), and the membrane was slightly cleaned by TBS and put into the milk solution, which was placed in a shaker for

shaking at room temperature for more than 4 hours. The dedicated primary antibody diluent was employed to prepare the corresponding dilution of the primary antibody, and the dilution ratios of TRPS1, P-gp, and  $\beta$ -actin were 1:400, 1:500, and 1:2000, respectively. PVDF membrane was placed in an antibody and incubated overnight at 4°C. The primary antibody was washed by TBST for 10 minutes / 3 times. The corresponding secondary antibodies were diluted to 1:400 with TBST, and the corresponding PVDF membranes were incubated at 37°C for 40 minutes. TBST was used for washing 10 minutes / 3 times; the electrochemiluminescence (ECL) solution was dropped to the PVDF membrane in the darkroom, which was covered with plastic wrap; X-ray film was for exposure, the development time was controlled, and there was a fixing after the film strip was clear and satisfied. After the fixed film was washed and dried, the image was scanned, and the brightness of the strip was quantitatively analyzed by computer software.

### Statistical analysis

All experiments were repeated at least 3 times under the same conditions, and the data of each group were expressed as mean  $\pm$  standard error ( $X \pm \text{sem}$ ). Pair comparison between groups was completed by f test, pair-for-pair comparison between multiple samples was completed by one-way analysis of variance, and the related processing of all data was completed by statistical software such as Fflowjo and GraphPad Prism 5.

## Results

### Detection results of qPCR and WB

The results of qPCR and WB were shown in Figure 1, in which A represented the result of qPCR and B stood for the result of WB detection. The expression levels of U2-OS and MNNG/HOS C1#5 were higher than the levels of Saos-2 cell lines. The MDR1 gene product was also expressed in U2-OS and MNNG/HOS C1#5, and P-gp expression was not detected in the Saos-2 cell line.

### Expression of TRPS1 and MDR1 genes in clinical bone tumor samples

The expression of TRPS1 and MDR1 in human osteosarcoma tissues was presented in Figure 2, suggesting that TRPS1 and MDR1/P-gp showed differences in the immunohistochemistry of different tumor centers in the human body. Positive TRPS1 meant staining of the nucleus, and positive MDR1 showed staining of the cell membrane. If both were positive, the tissue was from the same patient sample, and the tissue sample that was both negatives was also from the same patient. The specific analysis of the correlation between the two was shown in Table 4, and

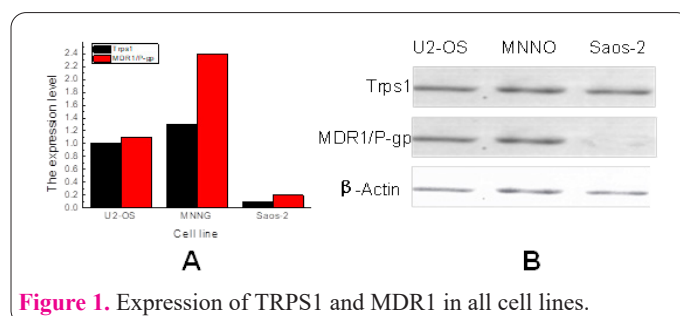
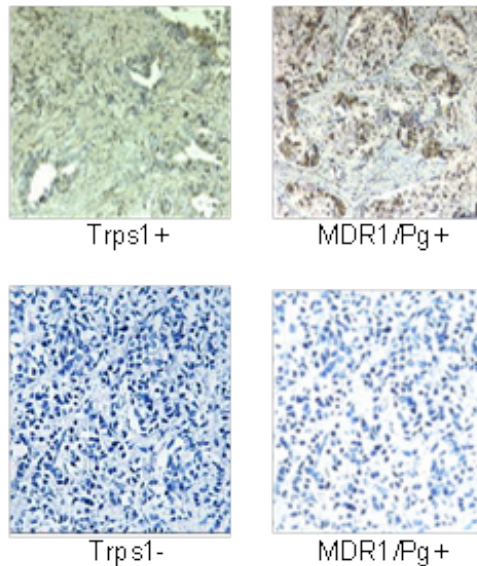


Figure 1. Expression of TRPS1 and MDR1 in all cell lines.

**Table 4.** Correlation analysis between TRPS1 and MDR1.

MDR1/P-gp	N	TRPS1score		P	Spearman correlation coefficient	Value (r)	P
		<1	≥1				
Total							
Negative	63	49	15	<0.001 <sup>b</sup>		0.477	<0.001
Positive	13	3	10				

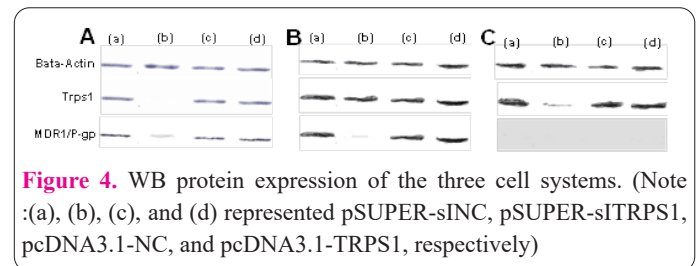


**Figure 2.** Gene expression of TRPS1 and MDR1 in human osteosarcoma.

could inhibit the expression of TRPS1 and MDR1/P-gp proteins in various cells.

**The cell drug sensitivity test results of the MTT assay**

Taking doxorubicin as an example, cell survival curves of each system in each group were drawn, as shown in Figure 6. It indicated that the growth curve of cells after transfection with miR-138 decreased obviously. There was no marked change in the cell growth curve of the control plasmid compared with the original curve. The transformation trend of the cell growth curve in each system of other drug cells was similar to that of doxorubicin, which would not be shown and described here.



**Figure 4.** WB protein expression of the three cell systems. (Note: (a), (b), (c), and (d) represented pSUPER-sINC, pSUPER-sITRPS1, pcDNA3.1-NC, and pcDNA3.1-TRPS1, respectively)

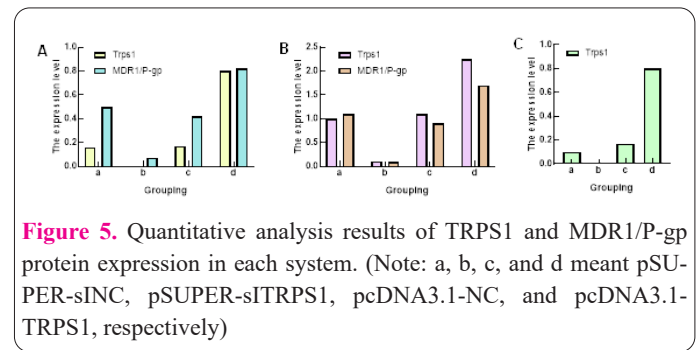
there was a strong positive correlation between them.

**mRNA expression of TRPS1 and MDR1/P-gp after plasmid transfection**

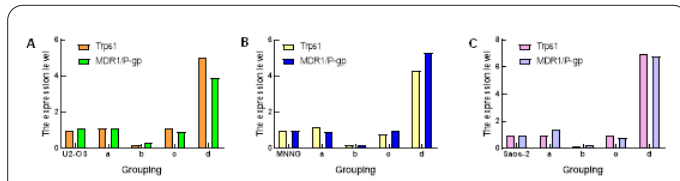
The mRNA expression of MDR1/P-gp in each cell line 48 hours after mi-RNA transfection was shown in Figure 3. What's more, a stood for the expression of U2-OS, B expressed the expression of the MNNG system, and C represented the expression of the Saos-2 system. Compared with the negative control group in each system, the expression level of TRPS1 in the mi-RNA interference group decreased, and the MDR1/P-gp mRNA reduced to 1/3-1/7 of the original level. In the over-expressed group, MDR1 levels increased by 4.2-8.4 times.

**Expression of TRPS1 and MDR1 proteins**

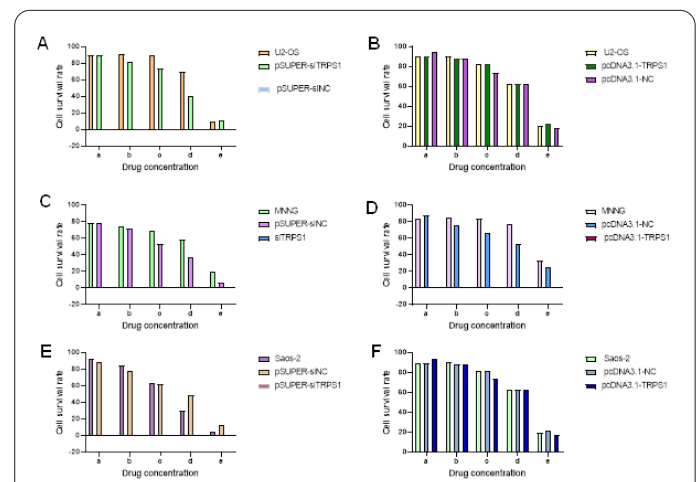
The protein expressions of TRPS1 and MDR1 were displayed in Figures 4 and 5. Figure 4 showed the WB protein expression test results of the three cell systems, which employed β-actin as an internal reference. Figure 5 was obtained by quantitative analysis using the classic software Image J for the gray level of protein strips. According to Figures 4 and 5, the transfection of mi-RNA



**Figure 5.** Quantitative analysis results of TRPS1 and MDR1/P-gp protein expression in each system. (Note: a, b, c, and d meant pSUPER-sINC, pSUPER-sITRPS1, pcDNA3.1-NC, and pcDNA3.1-TRPS1, respectively)



**Figure 3.** mRNA expression of TRPS1 and MDR1/P-gp after transfection with plasmid. (Note: a, b, c, and d stood for pSUPER-sINC, pSUPER-sITRPS1, pcDNA3.1-NC, and pcDNA3.1-TRPS1, respectively)



**Figure 6.** Doxorubicin sensitivity test results of each group. (Note: The drug concentrations corresponding to a, b, c, d, and e were 0, 0.04, 0.2, 1, and 5 ng/mL in turn; A and B indicated the cell survival conditions of each U2-OS cell line group; C and D meant the cell survival conditions of each MNNG cell line group; E and F showed the cell survival conditions of each Saos-2 cell line group.)

## The effect of miR-138 on the formation of bacterial biofilm

The results of counting live bacteria by ultrasonic oscillation were shown in Figure 7. Among them, ATCC29213 was *Staphylococcus aureus* and 3-35 was Methoxylin-resistant *Staphylococcus aureus*. The numbers of live bacteria of the two strains of SA in the miR-138 interference group were about 104 cfu/cm<sup>3</sup>-102 cfu/cm<sup>3</sup>. However, the numbers of two SA strains in the non-mi-RNA interference group were about 106 cfu/cm<sup>3</sup>, and the difference between the two was statistically substantial.

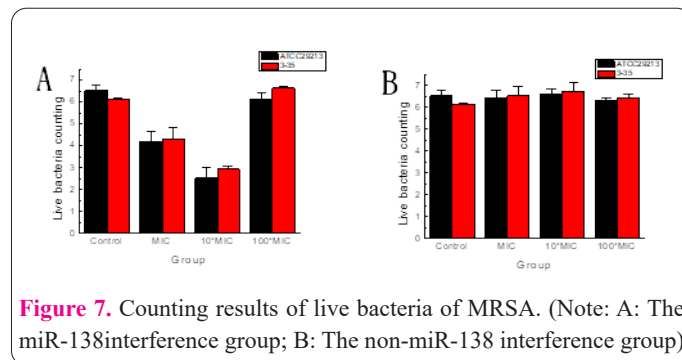
## Discussion

Osteosarcoma is a common malignant bone tumor originating from the stroma, which is characterized by easy metastasis, a high degree of malignancy, and young age of onset. The research data have shown that about 90% of patients with osteosarcoma die of pulmonary metastasis before chemotherapy is available. It can be said that osteosarcoma has long been a threat to human health (13).

Before the 1970s, the primary treatment for osteosarcoma was surgical resection, which resulted in poor survival and poor prognosis in patients with poor treatment. The rise of chemotherapy has led to a dramatic improvement in the treatment of osteosarcoma, with a 30% increase in the 5-year survival rate. Although osteosarcomas are tumors of the mesenchymal system, radiotherapy sensitivity is inherently poor. However, with the development and progress of tumor radiation therapy in recent years, the clinical control rate of osteosarcoma has also achieved a high. This makes tumor chemotherapy become one of the important means for the treatment of osteosarcoma. Currently, for the treatment of osteosarcoma, the prognosis is still not very optimistic due to multiple combined therapies (14).

Current treatments for osteosarcoma are based on mycin, cisplatin, and high doses of methotrexate. However, these drugs can be pumped by the P glycoprotein on the cell membrane, which leads to a significant increase in drug resistance of osteosarcoma and limits the success of chemotherapy. A large number of studies have shown that MDR1 can encode the expression of P glycoprotein (15,16). It is also found that TRPS1 can inhibit TGFβ-1 expression in mouse embryonic kidney tissue and suppress the TGFβ signaling pathway. Besides, it also has been reported that both TGFβ-1 and TGFβ-2 can inhibit the MDRL expression in human gliomas. It is suggested that TRPS1 can further affect the drug resistance of osteosarcoma cells by controlling the expression of p-glycoprotein by controlling MDRL (17). The results of this study were consistent with the above view. In other words, there was a positive correlation between the expression of TRPS1 and the MDRL gene, and the overexpression of TRPS1 would lead to the increased expression of P glycoprotein and improve the drug resistance of osteosarcoma tumor cells.

Although mi-RNA is a non-coding RNA, it influences the degradation and translation of mRNA. At present, the most studied mi-RNAs are miR-124, miR-363, miR-138, etc. The research data have pointed out that miR-138 has a certain inhibitory effect on TRPS1 based on the close relationship between TRPS1 and MDR1 (18). This research could further deduce that miR-138 could inhibit MDR1, thus further reducing the drug resistance of osteosarcoma. In this study, it was found that compared with bone tumor



**Figure 7.** Counting results of live bacteria of MRSA. (Note: A: The miR-138 interference group; B: The non-miR-138 interference group)

cells that did not express miR-138, tumor cells that expressed miR-138 had lower expression levels of TRPS1 and MDR1 and a lower drug resistance rate. This suggested that miR-138 could further affect the drug resistance of osteosarcoma tumor cells by regulating the expression of MDR1 through the TRPS1 signaling pathway. In addition, the researchers have also pointed out that miR-138 has a reliable influence on the transcription factor Rbf, which determines the formation of extracellular PLA and extracellular proteins. Exopolysaccharides and extracellular proteins are important components of biofilm. Therefore, it can be inferred that miR-138 also has an effect on the formation of biofilm (19). As a malignant tumor, osteosarcoma inevitably requires some invasive examinations during its treatment. These invasive examinations often lead to the formation of bacterial biofilms that can lead to refractory infections. This is very unfavorable for the treatment of osteosarcoma (20). Thus, it is necessary to control the formation of biofilm in the treatment of osteosarcoma. In this study, an in vitro biofilm formation model was established, and the influence of miR-138 on biofilm formation was explored. The results of this study revealed that compared with the non-miR-138 intervention group, the biofilm formation rate of the miR-138 intervention group was lower and the number of colonies was less. It indicated that miR-138 had a certain inhibitory effect on the formation of biofilm. Therefore, the results of this study could provide a new idea for the solution of drug resistance and refractory infection during the treatment of osteosarcoma. Furthermore, the specific mechanism of mi-RNA reducing the drug resistance rate of osteosarcoma cells and affecting biofilm formation still needs to be further explored.

TRPS1 was interfered with by mi-RNA, and the expression of MDR1, P-gp protein, the formation of *Staphylococcus* biofilm, and cell drug resistance was explored in this study. It was found that mi-RNA could further control the formation of cell-resistant *Staphylococcus* biofilms through TRPS1. The results of this study could provide a basis for clinically improving the drug resistance of osteosarcoma cells and the formation of biofilms. However, this study is not comprehensive and in-depth due to the limited sample and space.

## Fundings

The research is supported by: the 2020 Shaoguan City Science and Technology Plan Project (No. 200812214534164).

## References

1. Elster D, Tollot M, Schlegelmilch K, Ori A, Rosenwald A, Sahai



- E, von Eyss B. Author Correction: TRPS1 shapes YAP/TEAD-dependent transcription in breast cancer cells. *Nat Commun* 2018; 9(1): 3781. <https://doi.org/10.1038/s41467-018-06266-2>
2. Cornelissen LM, Drenth AP, van der Burg E, de Bruijn R, Pritchard CEJ, Huijbers IJ, Zwart W, Jonkers J. TRPS1 acts as a context-dependent regulator of mammary epithelial cell growth/differentiation and breast cancer development. *Genes Dev* 2020; 34(3-4):179-93. <https://doi.org/10.1101/gad.331371.119>
  3. Cho KY, Kelley BP, Monier D, Lee B, Szabo-Rogers H, Napierala D. Trps1 regulates development of craniofacial skeleton and is required for the initiation of palatal shelves fusion. *Front Physiol* 2019; 10: 513. <https://doi.org/10.3389/fphys.2019.00513>
  4. Witwicki RM, Ekram MB, Qiu X, Janiszewska M, Shu S, Kwon M, Trinh A, Frias E, Ramadan N, Hoffman G, Yu K, Xie Y, McAllister G, McDonald R, Golji J, Schlabach M, deWeck A, Keen N, Chan HM, Ruddy D, Rejtar T, Sovath S, Silver S, Sellers WR, Jagani Z, Hogarty MD, Roberts C, Brown M, Stegmaier K, Long H, Shivdasani RA, Pellman D, Polyak K. TRPS1 is a lineage-specific transcriptional dependency in breast cancer. *Cell Rep* 2018; 25(5): 1255-67. <https://doi.org/10.1016/j.celrep.2018.10.023>
  5. Yu SP, Yan RH, Gao J, Song D. Significance of TRPS1 in the development and clinicopathologic of hepatocellular carcinoma. *Eur Rev Med Pharmacol Sci* 2020; 24(18): 9325-32. [https://doi.org/10.26355/eurrev\\_202009\\_23014](https://doi.org/10.26355/eurrev_202009_23014)
  6. Hu J, Zhang H, Liu L, Han B, Zhou G, Su P. TRPS1 confers multidrug resistance of breast cancer cells by regulating BCRP expression. *Front Oncol* 2020; 10: 934. <https://doi.org/10.3389/fonc.2020.00934>
  7. Goss M, Socorro M, Monier D, Verdels K, Napierala D. Trps1 transcription factor regulates mineralization of dental tissues and proliferation of tooth organ cells. *Mol Genet Metab* 2019; 126(4): 504-12. <https://doi.org/10.1016/j.ymgme.2019.01.014>
  8. Penolazzi L, Lambertini E, Scussel Bergamin L, Gandini C, Musio A, De Bonis P, Cavallo M, Piva R. Reciprocal regulation of TRPS1 and miR-221 in intervertebral disc cells. *Cells* 2019; 8(10): 1170. <https://doi.org/10.3390/cells8101170>
  9. Liu Y, Xu S, Lian X, Su Y, Zhong Y, Lv R, Mo K, Zhu H, Xiaojiang W, Xu L, Wang S. Atypical GATA protein TRPS1 plays indispensable roles in mouse two-cell embryo. *Cell Cycle* 2019; 18(4): 437-51. <https://doi.org/10.1080/15384101.2019.1577650>
  10. Hu J, Su P, Jiao M, Bai X, Qi M, Liu H, Wu Z, Sun J, Zhou G, Han B. TRPS1 suppresses breast cancer epithelial-mesenchymal transition program as a negative regulator of SUZ12. *Transl Oncol* 2018; 11(2): 416-25. <https://doi.org/10.1016/j.tranon.2018.01.009>
  11. Sha J, Xia L, Han Q, Chi C, Zhu Y, Pan J, Huang Y, Xia W, Dong B, Xue W, Yang C. Downregulation of circ-TRPS1 suppressed prostatic cancer prognoses by regulating miR-124-3p/EZH2 axis-mediated stemness. *Am J Cancer Res* 2020; 10(12): 4372-85. <https://doi.org/10.21203/rs.3.rs-48783/v1>
  12. Zhang Y, Nakamura T, Furukawa F, Muragaki Y. Trps1-deficient transplanted skin gave rise to a substantial amount of hair: Trps1 is unnecessary for hair development. *Dermatol Reports* 2019; 11(1): 7853. <https://doi.org/10.4081/dr.2019.7853>
  13. Liu H, Liao Y, Tang M, Wu T, Tan D, Zhang S, Wang H. Trps1 is associated with the multidrug resistance of lung cancer cell by regulating MGMT gene expression. *Cancer Med*. 2018; 7(5): 1921-32. <https://doi.org/10.1002/cam4.1421>
  14. Zhang J, Ma X, Zhou R, Zhou Y. TRPS1 and YAP1 regulate cell proliferation and drug resistance of osteosarcoma via competitively binding to the target of circTADA2A - miR-129-5p. *Onco Targets Ther* 2020; 13: 12397-407. <https://doi.org/10.2147/OTT.S276953>
  15. Socorro M, Shinde A, Yamazaki H, Khalid S, Monier D, Beniash E, Napierala D. Trps1 transcription factor represses phosphate-induced expression of SerpinB2 in osteogenic cells. *Bone* 2020; 141: 115673. <https://doi.org/10.1016/j.bone.2020.115673>
  16. Li S, Chen Z, Yang Y. Novel mutation of TRPS1 in a patient with tricho-rhino-phalangeal syndrome. *Clin Exp Dermatol* 2021; 46(3): 557-9. <https://doi.org/10.1111/ced.14430>
  17. Serandour AA, Mohammed H, Miremadi A, Mulder KW, Carroll JS. TRPS1 regulates oestrogen receptor binding and histone acetylation at enhancers. *Oncogene* 2018; 37(39): 5281-91. <https://doi.org/10.1038/s41388-018-0312-2>
  18. Zepeda-Mendoza CJ, Cousin MA, Basu S, Jenkinson G, Oliver G, Pittcock ST, Baughn LB, Klee EW, Babovic-Vuksanovic D. An intragenic duplication of TRPS1 leading to abnormal transcripts and causing trichorhinophalangeal syndrome type I. *Cold Spring Harb Mol Case Stud* 2019; 5(6): a004655. <https://doi.org/10.1101/mcs.a004655>
  19. Wang H, Huang Y, Yang Y. LncRNA PVT1 regulates TRPS1 expression in breast cancer by sponging miR-543. *Cancer Manag Res* 2020; 12: 7993-8004. <https://doi.org/10.2147/CMAR.S263383>
  20. Wang C, Xu Y, Qing Y, Yao R, Li N, Wang X, Yu T, Wang J. TRPS1 mutation detection in Chinese patients with Tricho-rhino-phalangeal syndrome and identification of four novel mutations. *Mol Genet Genomic Med* 2020; 8(10): e1417. <https://doi.org/10.1002/mgg3.1417>