Preparation of the magnetically-targeting complex pHSP-Plk1-shRNA / DOX based on magnetotactic bacterial magnetosomes and study of osteosarcoma cell proliferation inhibition

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Abstract: Osteosarcoma is a common primary malignant bone tumor. At present, chemotherapy has certain toxic and side effects, thus it is of great significance to pursue new and effective alternative techniques. In this study, a novel drug-carrying magnetic targeting complex was prepared using magnetotactic bacterial magnetosomes as carrier, including doxorubicin (DOX), magnetotactic bacterial magnetosomes and plK1-shrNA eukaryotic expression plasmid with HSP70 heat shock promoter. The results of drug release tests showed that pHSP-PLK1-shRNA/DOX/BM compounds have a good in-body release potential without obvious instant releases condition. The proliferation activity of tumor cells was determined by MTT assay, the compound could inhibit the proliferation of osteosarcoma U2OS cells, especially under pulsed magnetic field exposure. The preparation of the complex provides a new possible clinical application approach and reference for magnetic targeted comprehensive therapy of osteosarcoma.

1. Introduction

Polo-like kinase 1(PLK1) is a serine/threonine protein kinase widely existing in eukaryotic cells with highly conserved structure and function. It is mainly involved in the activation of Cyclin B/CDK1 complex [1], assisting the functional maturation of centrosomes, activating anaphase promoting complex (APC) at the later stage of cell division, and promoting normal chromosome separation, allocation and cytoplasm separation, etc. [2]. Studies have found that this kinase is overexpressed in a variety of tumors, including osteosarcoma [3], and the overexpression of PLK1 is a marker of bleak prognosis of a variety of tumors. Therefore, the study of PLK1 is of great significance for tumor diagnosis, treatment and drug development [4]. On the other hand, in cancer clinical comprehensive therapeutics, thermal remedy has become an important method. The study found that the tumor tissues heated to a temperature of about 42℃ can kill tumor cells [5], and thermal remedy can effectively relieve symptoms, improve the condition of the patient's body, strengthen the body's immune function and thus enhance patients with disease resistance. In recent years, hyperthermia is often combined with chemotherapy or gene therapy to enhance its killing effect on tumor cells, the main theoretical basis of which is that heating can significantly enhance the tumor killing effect of some chemotherapy drugs [6,7]. In this study, a novel magnetic targeting complex was prepared using magnetotactic bacterial magnetosomes as carrier, including doxorubicin (DOX), magnetotactic bacterial magnetosomes, plK1-shrNA eukaryotic expression plasmid with HSP70 heat shock promoter. By detecting its effects on the proliferation of human osteosarcoma U2OS cells, the possible clinical application potential of the novel complex was evaluated.

2. Materials and methods

2.1 Culture and measurement of magnetosomes

Magnetotactic bacteria *magnetospirillum magneticum* AMB-1 was cultured on liquid bottle for 4 d at 30℃. Absorbance value of the magnetotactic bacteria was measured at wavelength 600 nm to calculate the growth development. The horizontal magnetic and vertical magnetic field were applied around the bacteria liquid. The absorbance value under the horizontal magnetic field was divided by the absorbance value under the vertical magnetic field, i.e., the magnetic coefficient C_{mag} with different light scattering conditions.

2.2 Purification and effect assay of magnetosomes

The collected bacteria were suspended in the PBS buffer at a ratio of 1:10 (mass: volume). They were disrupted for 3~4 times by a high pressure homogenizer (Pressure 120~140MPa). Magnetic separation system was used to separate magnetosomes and cell fragments, accompanied by low-power ultrasonic percussion. After the supernatant was basically transparent, a magnet was used to adsorb and discard the supernatant, and then the precipitation was suspended in PBS buffer. The supernatant was slightly untrasonicated by ultrasonication (80W, working for 4s, intermittent for 6s, 30 ~ 60 cycles), and the magnet was used to adsorb and repeat this step until the supernatant was utterly transparent. The OD280 and OD260 values of the supernatant were measured by a spectrophotometer. According to the empirical formula of protein content (mg / ml) = (1.45×OD280-0.74×OD260) × sample dilution, the protein content in the supernatant was estimated. The supernatant was washed three times with sterile deionized water to remove salt ions, and finally vacuumed freeze-drying to obtain the magnetosomes. The magnetosomes were made into a 2mg/ml suspension, and ultrasonically dispersed for 10 min to reduce particle agglomeration. The dispersed particle suspension was slightly adsorbed to a copper mesh, air-dried, and directly observed under a transmission electron microscope.

2.3 Preparation and drug release experiment of pHSP-Plk1 - shRNA / DOX complex

The HSP70 promoter sequence was obtained by PCR using the genome of human osteosarcoma cell line MG-63 as the template. The dual fluorescence detection system was used to verify whether the sequence had promoter activity in osteosarcoma cells, and the deletion analysis was used to confirm the core region of HSP70 promoter. The core region of HSP70 promoter was amplified by PCR, and the recombinant plasmid of Plk1-shRNA with HSP70 promoter was constructed by double digestion. At the same time, a shRNA fragment with different sources from human and mouse genes was designed and synthesized as a negative control (NC). The above suspension was centrifuged, redissolved in PBS buffer, and incubated with crosslinking agent genipin, pHSP-Plk1 - shRNA and adriamycin (DOX). After stirring at room temperature, the pHSPPlk1 - shRNA / DOX complex was obtained. Based on the characteristic absorption peak of DOX, the absorption curve and curve equation of DOX were determined. At the target wavelength, the absorbance of the complex at a certain interval was measured, and the release curve of DOX was drawn up

2.4 Determination of tumor cell proliferation activity under pulsed magnetic field by MTT assay

The tetramethylazolyl blue (MTT) colorimetric method was used. U2OS osteosarcoma cells in logarithmic growth phase was taken to make single cell suspension, and the concentration was adjusted to 4.0×10^4/ml. 200 μl per well of either the control group, magnetosomes (BMs) treatment group, DOX treatment group, pHSP-Plk1-shRNA transfection group, complex pHSP-NC/DOX/BMs group (negative control) or pHSP-PLK1-shRNA/DOX/BMs groupwas injected in 96-well plate, and the cells were treated with alternating pulsed magnetic field (50 Hz, 20 mT) for 1h. After 24h incubation, 20μl of 5mg / ml MTT was added to each well and incubated at 37 °C for 4h. The supernatant was then discarded, and 100μl dimethyl sulfoxide was added into each well. After shaking for 10 min, the blue crystal was fully dissolved, and the absorbance value was measured at wavelength 570 nm by enzyme-

linked immunosorbent assay. Cell growth inhibition rate (IR) = (control group absorbance value - experimental group absorbance value) / control group absorbance value × 100 %.

2.5 Statistical Analysis

SPSS18.0 software was used for statistical analysis of the data. The data were expressed as $\bar{x} \pm s$. One-way analysis of variance was used. $P < 0.05$ indicated the significant difference and $P < 0.001$ indicated extremely significant difference. GraphPad Prism5 software was used for graphing.

3. Results

3.1 Identification of purified magnetic bodies

The magnetosomes of *Magnetospirillum magneticum* AMB-1 were purified and prepared into samples for observation under a transmission electron microscope. The results showed that the magnetic particles were relatively uniform, with a size of about 50nm. The structure of biolipid membrane could be seen under the transmission electron microscope (Figure 1).

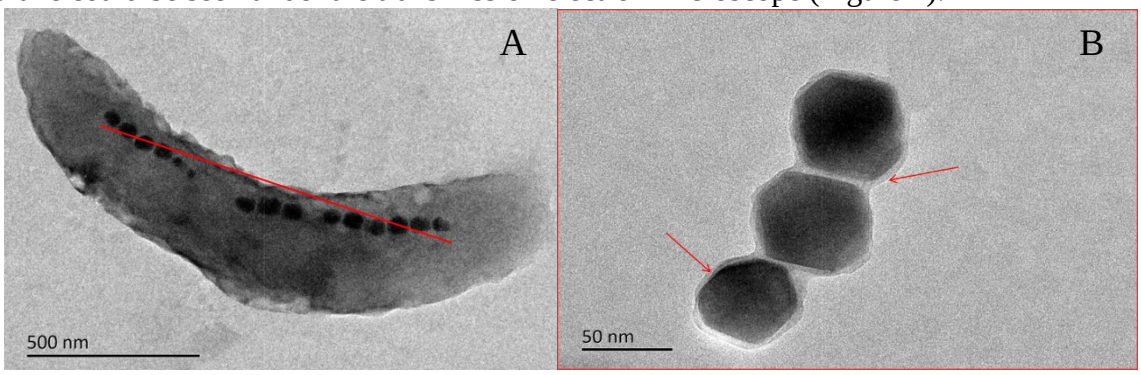

Figure 1 Magnetotactic bacteria AMB-1 and its magnetosomes (A) Magnetotactic bacteria AMB-1, the red straight line indicates the magnetosomes chain (B) Bacterial magnetosomes, the red arrow indicates the structure of bio-lipid membrane.

3.2 Release curve of compounds

It can be seen from Figure 2 that the free DOX is almost completely dissolved within 10 h at 37°C. Among the DOX prepared with different cross-linking reaction agents, however, the average release of DOX from the complex DBMs was less than 15% within 10 h, and there was no significant burst release within the initial half hour.

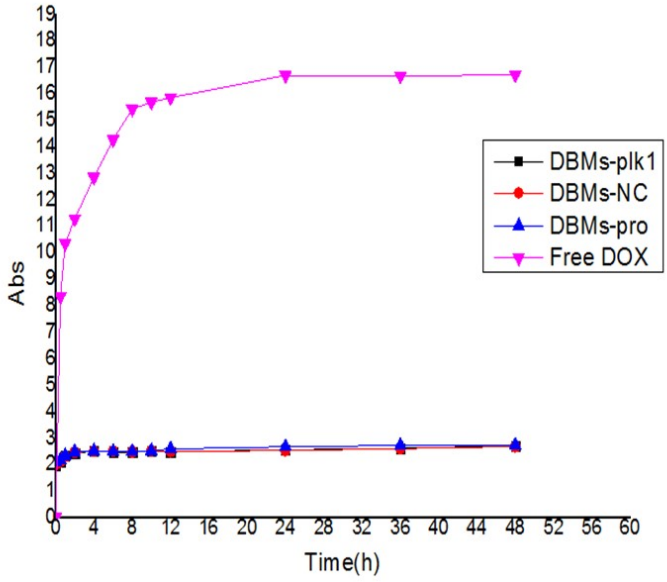

Figure 2 Determination of DOX release curve

3.3 Detection of proliferation activity of tumor cells under magnetic field treatment

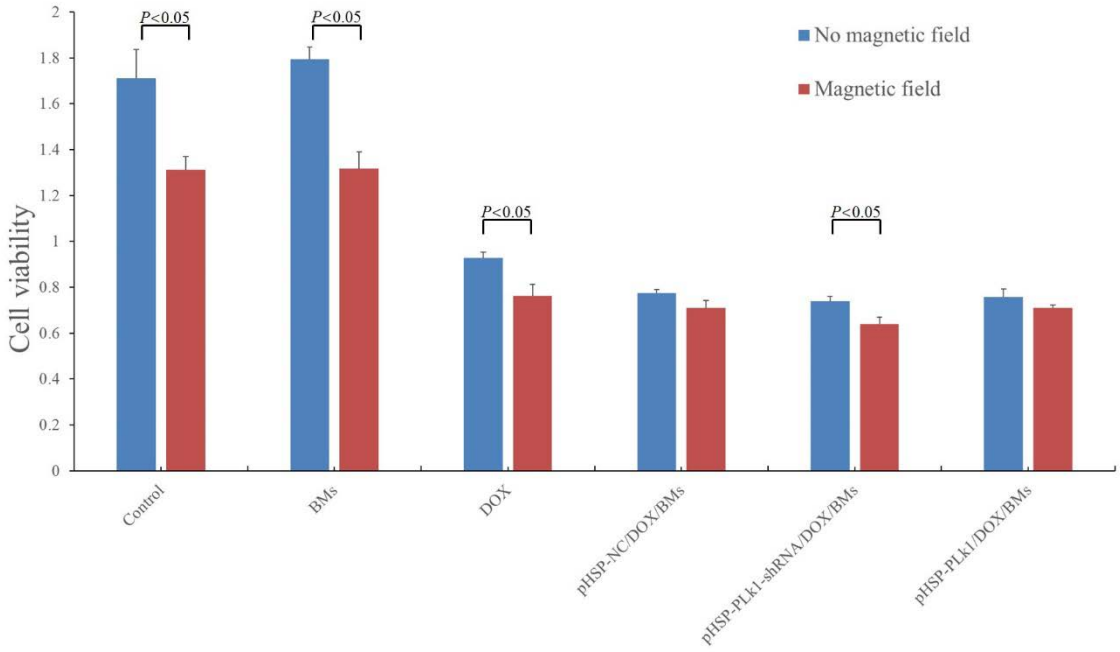

Figure 3 Tumor cell viability with groups of treatments by MTT assay

As seen from Figure 3, compared with the control, magnetosomes, DOX and pHSP-PLK1-shRNA/DOX/BMs complex all have significant inhibitory effects on tumor cell proliferation (P<0.05), with pHSP-PLK1-shRNA/DOX/BMs complex showing the most remarkable inhibitory effects, particularly 38% inhibition under pulsed magnetic field exposure.

4. Discussion and conclusions

Because PLK1 plays an important role in cell cycle and DNA damage, its overexpression is considered a marker of many bleak tumor prognosis, making PLK1 an iedaltarget for tumor therapy [8].Detection and control of PLK1 expression is of great significance for tumor early diagnosis and prognosis prediction of tumor patients. Exploring the involvement of PLK1 in physiological and pathological processes and targeting it to find new anti-tumor drugs or methods has become one of the research hotspots nowadays.

In conclusion, this study used magnetotactic bacterial magnetosomes as carriers, combined with promoter HSPs to prepare a new magnetic targeting complex, including doxorubicin (DOX), magnetotactic bacterial magnetosomes, and Plk1-shRNA eukaryotic expression plasmid under HSP70 heat-shock activation. The release test showed that the complex had good release potential in vivo, and there was no apparent burst release phenomenon. MTT assay of tumor cell proliferation activity showed that the complex had significant inhibitory effects on the proliferation of osteosarcoma U2OS cells, especially under the pulsed magnetic field, suggesting that it may be due to the dual function of chemicals and thermal effects of magnetosomes under the exposure of high-frequency alternating magnetic field, which provides a novel possible clinical application and reference for the comprehensive remedy of osteosarcoma.

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