Original Article

Effect of miR-143 on proliferation of osteosarcoma cells under low dosage cisplatin

Dacheng Li1, Fei Zhang2, Wangjun Yan3

1Department of Orthopedic Surgery, Ningbo Mingzhou Hospital, Ningbo, Zhejiang, China; 2Department of Orthopedics, Ningbo Development Zone Center Hospital, Ningbo, China; 3Department of Bone Oncology, Changzheng Hospital, Second Military Medical University, Shanghai, China

Received August 10, 2015; Accepted October 24, 2015; Epub January 1, 2016; Published January 15, 2016

Abstract: Osteosarcoma is one primary malignant bone tumor that commonly occurs in children and teenagers. Its high malignancy, invasiveness and metastasis all lead to unfavorable prognosis even with combined treatment. Cisplatin is a common chemotherapy agent in treating various solid tumors including ovarian, pulmonary, nasopharyngeal, esophageal, thyroid cancers and osteosarcoma, but leaving its mechanism largely unclear. Micro RNA (miRNA) has been suggested to have abnormal expression in tumor tissues. This study thus observed the effect of miR-143 on the proliferation of osteosarcoma cell MG63, via the manipulation of miR-143 expression under the exposure of low dosage cisplatin. CCK8 assay was performed to observe the influence of cisplatin on MG63 cell proliferation to determine LC50 value of cisplatin. MG63 osteosarcoma cells were ten transfected with miR-143 mimic or miR-143 inhibitor to observe the altered cell proliferation under low concentration of cisplatin exposure. Cisplatin could inhibit proliferation of MG63 cells. Under low dosage cisplatin, expression of miR-143 increased. The manipulation of endogenous miR-143 level can affect the proliferation of osteosarcoma cells. The up-regulation of miR-143 can inhibit the proliferation of osteosarcoma. Under the exposure of low dosage of cisplatin, miR-143 may participate in the inhibition of tumor cell proliferation.

Keywords: Osteosarcoma proliferation, cisplatin therapy, microRNA

Introduction

As one common primary bone cancer, osteosarcoma is mainly developed in children and teenagers [1]. It frequently occurs in metaphysis of long bones such as femur, tibia and humerus, and presented as low differentiation and high invasiveness [2]. Due to the sufficient blood flow in metaphysis, blood-borne metastasis often occur in highly malignant osteosarcoma patients affecting critical organs including lung, brain and kidney [3]. Tumor patients with metastasis thus have unfavorable prognosis, causing heavy burdens for patients and their families [4]. The new generation of chemotherapy using cisplatin can significantly improve the 5-year overall survival rate to about 70% [5]. Cisplatin, as one kind of metal complex, has several advantages including wide spectrum, high efficacy and less cross-resistance during chemo-therapy, and has gained satisfactory effects in treating various tumors [6]. Epidemiology survey has confirmed treatment efficacy of cisplatin against various solid carcinomas including ovarian, pulmonary, nasopharyngeal, esophageal, thyroid cancer and osteosarcoma, but leaving its anti-tumor mechanism largely unknown [7].

MicroRNA (miRNA) has been known to play a critical role in the pathogenesis of various tumors [8]. As one kind of small non-coding RNA molecule with 18~22 nt length, miRNA is widely distributed in all eukaryotes from plants to human. Mature miRNA is edited from premiRNA by Dicer enzyme, after the transcription from miRNA gene under the direction of RNA polymerase II. It mainly regulates the expression of target genes and hence related biological functions via specific binding onto the 3’-untranslated region (UTR) of the target gene mRNA. Actually more than 30% of all genes in our body are mediated by miRNA family, which thus plays crucial roles in various processes including cell growth, proliferation, differentiation and apoptosis [9]. Meanwhile, miRNA has
MiR-143 and osteosarcoma

highly-conserved sequence and is related with occurrence of multiple tumors. Current studies have revealed the participation of miRNA in pulmonary, liver, gastric, colorectal, thyroid cancers and osteosarcoma as either tumor activator or inhibitor [10]. Therefore, the identification of tumor-specific miRNA may have critical implication for the early-diagnosis and treatment of tumors. Cisplatin, as one common chemotherapy drugs in clinics, has been found to affect miRNA expression in tumors. This study thus focused on the osteosarcoma-specific miRNAs under the exposure of lower dosage of cisplatin, in an attempt to investigate the role of miRNA in tumor proliferation.

Materials and methods

Cell culture

MG63 osteosarcoma cell line was purchased from Chinese Academy of Science (Shanghai, China) and was incubated in DMEM culture medium containing 10% fetal bovine serum (FBS, Gibco, USA). Cells were incubated in a humidified 37°C chamber bubbling with 5% CO₂ gas.

CCK assay

Cells were seeded into 96-well plate (0.5 × 10⁴ per well), which was incubated for 24 hours. Serially diluted cisplatin (1 μg/mL, 2.5 μg/mL, 5 μg/mL, 10 μg/mL, 25 μg/mL, 50 μg/mL, 75 μg/mL and 100 μg/mL) was added into each well along with blank control and DMSO control wells. Each dosage was replicated in 6 parallel wells. After 48-hour incubation, the medium was removed, followed by addition of 10% CCK8 reagent (Ddjindo, China). The plate was incubated for 4 hours, and was quantified by absorbance value at each well using a microplate reader (Bio-Tek, US). The LC₅₀ value was deduced based on the standard growth curve against cisplatin dosage.

Real-time fluorescent quantitative PCR

Total RNA was extracted by Trizol reagent (Invitrogen, US) following manual instruction. RNA products were denatured at 65°C for 5 min and were in vitro reverse transcribed to cDNA with the help of reverse transcription kit (Totyobo, Japan). Using cDNA as the template and specific primers (U6-F: 5’-GCTTC GGCAG CACAT ATACT AAAAT-3’; U6-R: 5’-CGCTT CACGA ATTTG CGTGT CAT-3’; miR-143-F: 5’-AGTCA GTGAG ATGAA GCACT G-3’; miR-143-R: 5’-GTGCA GGGTC CGAGG T-3’), qPCR was performed using SYBR green mix (Toyob, Japan) under the following conditions: 95°C pre-denature for 5 min, followed by 40 cycles each containing 95°C denature for 15 sec, 60°C annealing for 45 sec and 72°C elongation for 15 sec. Semi-quantitative analysis was performed by 2⁻ΔΔCt method.

Cell transfection

MG63 cells were firstly seeded into 96-well plate at 0.5 × 10⁴ per well density. After 24-hour incubation, cells were changed for serum-free medium, followed by Lipofectamine 2000 (Invitrogen, US) plus miR-143 mimics or inhibitors. After a brief incubation, complete DMEM medium was changed for further incubation. The proliferation status of cells was evaluated by CCK8 assay after 48-hour incubation.
MiR-143 and osteosarcoma

Statistical analysis

SPSS 19.0 software package was used to process all collected data, of which measurement data were presented as mean ± standard deviation (SD). Student t-test or analysis of variance (ANOVA) was used to compare means between groups. Enumeration data were presented as percentage and compared by chi-square test. All experiments were carried out in at least triplicates. A statistical analysis was defined when P<0.05.

Results

Toxicity of cisplatin on osteosarcoma cells

Under different concentrations of cisplatin (1 μg/mL, 2.5 μg/mL, 5 μg/mL, 10 μg/mL, 25 μg/mL, 50 μg/mL, 75 μg/mL and 100 μg/mL), we tested the proliferative ability of MG63 cells at 3 different treatment periods (24 hours, 48 hours and 72 hours). As shown in Figure 1, high dosage of cisplatin impaired the survival rate of cells. Meanwhile, elongated treatment of drug also compromised the proliferation of cells. The LC50 values of cisplatin were determined to be 41.5 μg/mL (24-hour treatment), 23.8 μg/mL (48-hour treatment) and 12.2 μg/mL (72-hour treatment).

Effect of cisplatin on miR-143 expression

Using 48 hours as the standard cell treatment period, we quantified expression level of miR-143 in MG63 cells under different dosages of cisplatin (1 μg/mL, 2.5 μg/mL, 5 μg/mL, 10 μg/mL, 25 μg/mL, 50 μg/mL, 75 μg/mL and 100 μg/mL). As shown in Figure 2, miR-143 expression was induced by cisplatin exposure in a dose-dependent manner and reaching a peak value around LC50 (25 μg/mL). When the concentration of cisplatin was higher than LC50, elevation of cisplatin interestingly decreased miR-143 expression to some extents. These results suggest the potentiation of miR-143 expression by low dosage cisplatin.

Osteosarcoma cell proliferation and miR-143

To illustrate the role of miR-143 in the proliferation of osteosarcoma cells under low dosage of cisplatin exposure, MG63 cells were transfected with miR-143 mimics or inhibitors, along with their respective controls, followed by DMSO or cisplatin (2.5 μg/mL) treatment 24 hours later. After 48 hours, CCK8 assay kit was used to identify the proliferation of cells. Cisplatin-treated osteosarcoma cells had depressed proliferative rate compared to DMSO control cells (Figure 3A). Those cells with miR-143-overexpression also had inhibited cell proliferation (Figure 3A). On the other hand, the inhibition of miR-143 effectively promoted the proliferation of tumor cells (Figure 3B). These results collectively suggest the involvement of miR-143 in the proliferation of osteosarcoma.

Discussion

As one common malignant bone tumor, osteosarcoma is featured with high malignancy and invasiveness, making the frequent complica-
MiR-143 and osteosarcoma

tion of distal metastasis including lung, brain and kidney [11]. Once the occurrence of metastatic lesion, surgery is usually not optimal treatment strategy, and gives the way to radio-/chemo-therapy [12]. Cisplatin has been used in treating various cancers including pulmonary carcinoma, osteosarcoma and liver cancer [13,14]. Unfavorable prognosis exists for osteosarcoma largely due to its lower differentiation and higher invasiveness. Cisplatin has been shown to have satisfactory treatment efficacy against osteosarcoma, although its mechanism needs to be further elaborated.

As one kind of non-coding small molecule RNA with highly conserved sequence, miRNA exerts its biological function via complete or incomplete base-paring with 3’ UTR of the target gene mRNA. Although only occupying 1% of total genomic length, miRNA can modulate about 30% genes of our body by its unique regulatory patterns [9]. A complex mediatory network thus can be formed by both convergence and divergence regulation patterns of miRNA [15]. Recent tumor studies have revealed the importance of miRNA in oncogenesis, making it as one research focus of targeting therapy against tumors. Abnormal expression of miRNA has been found in multiple tumors. For example, thyroid cancer cells had depressed miR-183 expression [16]. Liver cancer had elevated miR-34a levels [17]. Pulmonary carcinoma, also was shown to have miR-203 down-regulation [18]. MiR-143 was also found to be down-regulated in tumor tissues such as those in gastric, rectal cancer and osteosarcoma [19-21]. Due to the pluripotency of miRNA in gene regulation and inherent complex of tumor formation, miRNA may play oncogenic or tumor suppressor roles in oncogenesis [22]. To study the role of miR-143 in treating osteosarcoma by cisplatin, we detected the expression of miR-143 and tumor cell proliferation of MG63 under low dosage of cisplatin.

Our results showed that cisplatin can inhibit the proliferation of MG63 cells in a dose-dependent manner with a saturation level. LC₅₀ values of cisplatin were determined to be 41.5 μg/mL, 23.8 μg/mL, and 12.2 μg/mL for 24-hour, 48-hour and 72-hour treatment, respectively. We also found that, after 48-hour of cisplatin exposure, miR-143 expressed was increased with elevating dosage of cisplatin below LC₅₀ concentrations. The potentiation of miR-143 expression reached a peak around LC₅₀ value. With further higher cisplatin concentration, miR-143 expressed was, however, depressed. We further inhibited or over-expressed endogenous expression of miR-143 and found negative correlation between cell proliferation and miR-143 expression: Those cells with higher expression of miR-143 had lowered cell proliferative ability; while those with miR-143 silencing had higher cell proliferation. Based on these results, we proposed that miR-143 can inhibit the proliferation of osteosarcoma under low dosage of cisplatin exposure. Previous study has found the induction of anti-apoptotic factors Bcl-2 by miR-143, for accelerating tumor cell apoptosis [23]. Therefore, it is believed that under low dosage cisplatin treatment, miR-143 may be activated in induce the expression of Bcl-2, which further impede the cell proliferation. Due to the complex network regulating the occurrence of osteosarcoma, cisplatin may have complicated mechanisms governing its treatment efficacy, thus requiring further comprehensive studies.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Wangjun Yan, Department of Bone Oncology, Changzheng Hospital, Second Military Medical University, 28 Fuxing Road, Shanghai 200003, China. Tel: +86-21-81886777; Fax: +86-21-81886777; E-mail: yanwangjun898@sina.com

References

MiR-143 and osteosarcoma


