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Research Paper

Association of circulating miR-125b and survival in patients with osteosarcoma–A single center experience

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ABSTRACT

Background: It is known that miRNAs play various roles in malignant tumors. This study is designed to investigate whether miR-125b levels can be used to predict the clinical response of patients with osteosarcoma (OS) to cisplatin-based chemotherapy.
Methods: From January 2010 to July 2015, 82 patients with resectable OS and 56 patients with unresectable OS were enrolled. Blood samples were collected and quantitative real-time PCR was applied to determine miR-125b expression. Clinical data was collected through medical records, and patients were treated according to National Comprehensive Cancer Network guidelines on OS.
Results: Our study found that patients with low miR-125b expression had shorter disease-free survival ($p < 0.001$) in the OS group, which was verified by Kaplan-Meier analysis and univariate and multivariate Cox analyses ($p < 0.001$). For patients with unresectable OS, low miR-125b expression was found to be associated with advanced tumor stages ($p = 0.006$). No complete remission was observed, and there were 13 patients with partial remission, 21 with stable disease, and 22 with disease progression. Negative correlation was found between miR-125b expression and response to chemotherapy ($p < 0.001$, $r = -0.606$). Furthermore, ROC analysis indicated that miR-125b at the cut point of 0.61 yielded an area under the ROC curve of 0.793 ($p < 0.001$, 95% CI: 0.664–0.890) in distinguishing chemotherapy-resistant OS from chemotherapy-sensitive OS, with sensitivity and specificity at 76.9% and 79.1%, respectively. Kaplan-Meier analysis and univariate and multivariate Cox analyses showed that patients with low miR-125b expression suffered shorter overall survival ($p = 0.014$, $p = 0.024$, and $p = 0.049$, respectively).
Conclusion: Down-regulation of circulating miR-125b might have the potential to predict cisplatin-based chemotherapy resistance and poor prognosis in OS.

1. Introduction

Osteosarcoma (OS) is the most frequently diagnosed malignant bone tumor in children and adolescents, and about half of the cases have lesions localized in the distal femur and proximal tibia [1]. The incidence of OS is estimated to be about three to five cases/million/year, accounting for about 5% of childhood malignancies and about 9% of malignancy-related deaths in children [2,3]. OS has a high propensity to metastasize, especially to the lung [4]. The long-term survival rate of OS patients was less than 20% after surgical resection alone prior to the availability of neoadjuvant and adjuvant chemotherapy in the 1980s [5]. The 5-year survival rate improved to 60–70% after the development and use of multi-agent chemotherapy regimens [6]. However, the improvement of survival has not changed significantly for the past 30 years since chemotherapy was developed, and chemoresistance has become a troublesome obstacle during management of OS. There is an urgent need to elucidate the molecular mechanisms of chemotherapy resistance and find reliable biomarkers of its development. This would greatly help identify more effective biological-based therapies and optimize the treatment strategies.

Post-transcriptional regulation by microRNAs (miRNAs) has been identified as an important mechanism underlying oncogenesis, invasiveness, proliferation, and migration of malignant tumors [7,8]. Growing evidence indicates that various miRNAs (including miR-92a, miR-99b, miR-132, miR-193a-5p, miR-422a, and miR-125b) are involved in the development of resistance to chemotherapy [5,9,10]. It is thought that miR-125b can act as both an oncogene and a tumor suppressor, depending on the cellular context [11–13]. A previous study indicated that miR-125b was significantly reduced in OS tissues, and that it suppresses proliferation and migration of OS cells through down-regulation of

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STAT3 [14]. Another study showed that miR-125b increases the sensitivity of OS cell lines to cisplatin by targeting Bcl-2 [10]. However, evidence from clinical practice is lacking.

Therefore, this study was designed to explore the possible use of miR-125b levels in patients to predict the response of OS to cisplatin-based chemotherapy.

2. Patients and methods

2.1. Ethical considerations

The study protocol was approved by the medical ethics committee of the Second Clinical Hospital of Lanzhou University. Informed consent was obtained from all adult participants prior to the start of the study. For children under 18 years of age, informed consent was obtained from their legal guardian.

2.2. Patients and samples

Patients with OS who presented to our department between January 2010 and July 2015 were screened for enrollment. Exclusion criteria were previous malignant tumors in another organ or system; hematological disorders; end-stage patients not qualified for chemotherapy; patients with no pathological data; and any patients unwilling to participate. Patients were treated according to National Comprehensive Cancer Network practice guidelines for OS, and any treatment decisions were not affected by participation in the study. Patients with resectable OS received cisplatin-based neoadjuvant and adjuvant chemotherapy and surgery, while cisplatin-based aggressive chemotherapy was given to OS patients with unresectable lesions.

The demographic and clinical characteristics of the study participants were obtained from their medical records on admission, and follow-up of patients receiving surgery was performed by the combination of outpatient visits, letters, and telephone calls. The patients had follow-up visits with physical examinations and radiography every 3 months, as well as computed tomography scans or magnetic resonance imaging when necessary. Disease-free survival was calculated from the date of surgery until an event for each patient. For patients with unresectable OS tumors, the response to chemotherapy was evaluated by the Response Evaluation Criteria in Solid Tumors (RECIST). In this study, chemotherapy sensitivity was defined as complete remission or partial remission, whereas stable disease and disease progression were taken as signs of chemotherapy resistance [15]. The patients were also followed for overall survival after therapy, which was calculated from the date of the beginning of chemotherapy in our department, until the date of death.

2.3. Sample collection, RNA isolation and quantitative real-time PCR (qRT-PCR) analysis

In each participant, a 10 mL peripheral venous blood was collected in EDTA anticoagulation tubes before any therapy was begun. The blood samples were centrifuged at 3,000 rpm for 10 min within 20 min after collection. In order to completely remove the cellular debris, the supernatant was separated and further processed by 15 min of high-speed centrifugation at 12,000 x *g*. The final plasma was then stored in RNase-free tubes (Axygen, Union, CA) at −80 °C for further analysis.

Total RNA was extracted from specimens using a mirVana™ PARIS™ kit (Applied Biosystems, USA) according to manufacturer's protocol. A NanoDrop™ 1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA) was used to determine the concentration of extracted RNAs. Taqman® MicroRNA Reverse Transcription Kits (Applied Biosystems, Foster City, CA, USA) were then utilized to perform the reverse transcription reactions. A 5 μl reaction system comprising 0.5 μl of different primers, 0.063 μl of 20 units/μl RNase inhibitor, 0.33 μl of 50 units/μl Multiscribe reverse transcriptase, 0.05 μl of 100 mM dNTPs, 0.5 μl of 10 × reverse transcription buffer, the RNA sample, and RNase-free water was incubated at 30 °C for 10 min, followed by 30 min of incubation at 50 °C, at 95 °C for 5 min, and then held at 4 °C.

A Bio-Rad IQ5 (Bio-Rad Laboratories Inc.) thermocycler was applied for the qPCR reaction. A 10 μl qPCR reaction solution with 5 μl of TaqMan 2 × Perfect Master Mix, 2 μl of cDNA solution, 0.25 μl of specific primers, and 2.75 μl of RNase-free water was used. U6 snRNA (Ambion, AM30303) was used as a reference miR. The qPCR primers were miR-125b: sense, 5′-GCUCCCUGAGACCCUAAC-3′, and antisense, 5′-CAGTGCAGGGTCCGAGGT-3′; U6: sense, 5′-CTCGCTTCGGCAGCACATATACT-3′ and antisense, 5′-ACGCTTCACGAATTTGCGTGTC-3′. The PCR amplification was performed as follows: an initial denaturation at 95 °C was carried out for 2 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The reaction was terminated by incubation at 95 °C for 15 s, and the products held at 4 °C. The expression level of miR-125b was calculated using the ΔΔCT method, and each reaction was repeated in triplicate to avoid bias.

2.4. Statistical analysis

MedCalc for Windows, version 13.0 (MedCalc Software, Ostend, Belgium) and SPSS version 16.0 (SPSS, Chicago, IL, USA) were used for statistical analyses. The Kolmogorov-Smirnov test was applied to test the normality of miR-125b expression in both groups, and one-way analysis of variance testing, Student's *t*-test and Spearman correlation analysis were flexibly used as appropriate. Receiver operating characteristic (ROC) curve analysis was conducted to evaluate the efficacy of miR-125b in distinguishing chemotherapy resistance in the OS group with unresectable lesions. The association between miR-125b expression and survival was assessed by the log-rank test and Cox proportional hazard regression analysis, and age, gender, tumor location, tumor stage, histologic grade, metastasis status, and miR-125b expression level were entered into the multivariate analysis. The mean value of miR-125b expression level was set as the cut-off point to differentiate patients with high or low miR-125b expression in the resectable and unresectable groups. A level of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Patients' characteristics

From January 2010 to July 2015, 176 patients who presented with OS to our orthopedic department were screened. Of these, 138 (78%) were enrolled as participants in the study, 82 with resectable OS and 56 with unresectable OS. The median follow-up time in the resectable OS group is 23.1 ± 10.9 months, while it was 13.9 ± 6.6 months in the unresectable OS group. The details of patients' clinical characteristics are presented in Table 1.

3.2. miR-125b expression profile in the resectable OS group

The miR-125b expression level in the resectable OS group was normally distributed with a mean of 0.97 ± 0.55 ($p > 0.05$, Table 2), and no significant correlation was found between miR-125b expression and patients' age, gender, tumor location, tumor grade, histologic grade, number of metastases, or location (Table 2). The samples with miR-125b expression less than 0.97 were assigned to