Original Article
Low expression of miRNA-1290 associated with local invasion and recurrence in sacral chordoma

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Abstract: Chordoma is a rare, locally aggressive neoplasm of bone, usually with poor prognosis. The treatment for chordoma has been unsatisfactory for decades. MiRNAs were recently introduced into this field and provided new insights to the pathogenesis and pathophysiology of chordoma. However, molecular basis of chordoma remains ambiguous up to now. This research aims to discover novel miRNA molecules as potential biomarkers and therapeutic targets. We measured the expression of miRNA-1290 in chordoma tissues and fetal nucleus pulposus tissues by quantitative real-time PCR. Further, we analyzed its association with the clinical features as well as the prognosis of patients. The expression of miRNA-1290 in chordoma samples was significantly lower than fetal nucleus pulposus samples (P=0.026). Low expression of miRNA-1290 contributed to tumor invasion into surrounding muscle (P=0.013), while no obvious significance was identified between miRNA-1290 expression and patients’ age, gender, tumor location and size (P>0.05). Log-rank test showed that low-level miRNA-1290 expression had a prominent impact on the patients’ RFS (P=0.004). Conclusively, miRNA-1290 might be a valuable prognostic biomarker and efficient therapeutic target for sacral chordoma.

Keywords: Chordoma, nucleus pulposus, microRNA, invasion, recurrence, prognosis

Introduction
Chordoma is a primary malignant bone tumor arising from the embryonic notochordal remnants, which often occurs at axial skeleton, including sacral region, spinal and skull base [1]. The overall age-adjusted incidence rate of chordoma is 0.089 per 100,000 annually [2], while the sacral chordoma occurs in 0.03 out of every 100,000 people per year [3]. The sex distribution reflects an obvious differentiation that it is approximately 2 times more common in male than female [4]. The survival rate of 5 years is 65% and it drops to 35% when it comes to a decade [5]. Chordoma is highly recurrent, with an appalling rate ranging from 44% to 78% [6-10], making itself prone to malignancy in terms of clinical progression and outcome. Surgical resection is recommended as the most effective treatment. However, it is quite difficult to receive complete surgical resection due to the complexity of anatomy. Besides, even though high-dose radiotherapy achieves reasonable disease control in most cases, there still lacks sufficient chemotherapy for subsequent adjuvant treatment, which contributes to the poor prognosis due to the high risk of recurrence [11]. Up to now, few biomarkers has been explored to identify the clinical features and characteristics of chordoma. Therefore, finding biomarkers for target therapy and prognosis indication is of great significance.

MicroRNAs are small noncoding RNAs of about 22 nucleotides in length. They were usually considered as noises in gene regulators. However, as the evidences have been accumulating, these microRNAs are now thought to function as antisense regulators of other RNAs [12]. Previous studies have shown that plenty of miRNAs have different expression in a variety of cancers and the irregular level of them closely correlates with tumor characteristics [13-15]. Yet, little has been studied about the abnormally expressed miRNAs in chordoma. Our previ-
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Figure 1. HE staining of chordoma tissue (A-C: ×100, ×200, ×300).

ous research showed that the miRNA-1290 had great potential as a regulator in chordoma microenvironment [16]. More importantly, no one has ever reported its association with invasion and recurrence of chordoma. Hence, in this article, we explored the differential expression of miRNA-1290 between sacral chordoma and nucleus pulposus and furtherly evaluated its correlation with clinical features as well as prognosis.

Materials and methods

Patients and samples

Our study enrolled 28 patients (20 males and 8 females) from Jan. 2005 to Jun. 2015 at the First Affiliated Hospital of Soochow University (Suzhou, China), with their average age of 56.4 years (range 18-84 years) when they were admitted to hospital. All the patients received primary surgical treatment and have received no adjuvant therapy preoperatively. Meanwhile, to settle the control group, 10 nucleus pulposus tissues were collected from aborted fetus at obstetric out-patient clinic of our hospital, with average age of 20.6 months (range 12-28 months). The samples were verified by means of histopathology (the representative histological figure of chordoma is shown as Figure 1) and preserved in liquid nitrogen. All the patients or families are informed of standard written consent. Our study protocol was approved by the Ethics Review Committee of the Institutional Review Board of the hospital.

Total RNA extraction and quantitative real-time PCR

Total RNA was extracted from 0.1 cm³ frozen tissue with TRizol reagent (TAKARA BIO INC., Shiga, JAPAN). cDNA was reverse transcribed from total RNA samples by RT primers of TaqMan MicroRNA Assays (Applied Biosystems, Foster City, USA) and TaqMan Small RNA Assay was equipped to operate the amplified reaction according to the manufacturer’s instructions (Applied Biosystems, Foster City, USA). U6 was used as internal control. The primers sequences were as follows: miR-1290, forward 5'-CAGT-GCTGGATTGTTGAT-3', reverse 5'-TATGGTTGGT-CACGACTCTTCAC-3'; U6, forward 5'-ATTGG-AACGATACAGAGAATT-3', reverse 5'-GGAAC-GCTTCACGAATTTG-3'. Each operation was repeated for three times. The C_t value for miRNA-1290 was normalized to U6 snRNA and the relative expression of miRNAs was calculated by the 2^-ΔΔCt method. Patients were divided into two groups depending on the average of C_t value.

Clinical data collection and follow-up

All the clinical data including age and gender of the patients as well as the size and location of tumors were collected. Largest diameter recorded in millimeters was used to represent the tumor size. As to tumor location, tumors located above S3 were regarded as one group while tumors located cephalad to S3 was considered as the other. Magnetic resonance imaging (MRI) was performed before surgery to assess tumor invasions into surrounding muscles. For all patients who have undergone surgery, plain radiographs, computed tomography scans (CT) and MRI are acquired every 3-6 months. Then assessment of which evaluated the tumor recurrence and postoperative life condition was made by imaging examination along with clinical manifestations. Recurrence free survival time (RFS) is defined as a time period dating from the primary surgery to tumor recurrence. All patients were followed up well for over 24 months. Disease free survival at the end of follow-up or lost to follow-up was regarded as censored data. Two typical cases were attached as Figures 2 and 3 displayed.

Statistical analysis

Statistical analysis was performed by SPSS 22.0 software (SPSS Inc., Chicago, IL). All the measurement data was is expressed by mean ± standard deviation (±). Shapiro-Wilk test
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was used to classify the measurement data into normal or abnormal distribution, then student’s t-test or Mann-Whitney U test will be furtherly adopted respectively, along with chi-square analysis to evaluate the correlation between miRNA-1290 expression and clinical features of patients. Kaplan-Meier survival curves and log-rank test were used to assess the association between miRNA-1290 expression and RFS. P<0.05 was considered statistically significant.

Results

The expression of miRNA-1290 in chordoma samples was significantly lower than fetal nucleus pulposus samples (P=0.026; Figure 4). The overall rate of tumor invasion was 60.7% (17/28). Notably, 77.8% (14/18) patients who had low miRNA-1290 expression occurred invasion while the rate in the opposite group was only 30% (3/10). Moreover, Chi-square analysis revealed that the low expression of miRNA-1290 contributes to tumor invasion (P=0.013; Table 1). In addition, no obvious significance was identified between miRNA-1290 expression and patients’ age, gender, tumor location and size.

Over an average span of 77.5±18.52 months (ranged from 25 to 139 months), satisfactory follow-up was achieved in all patients. The total recurrence rate was 57.1%, for local recurrence happened in 16 of the 28 patients. The median relapse time was 31.4±6.73 months. Within all the patients with low miRNA-1290 expression, 77.8% (14/18) had a tumor recurrence. However, only 20% (2/10) patients who expressed low miRNA-1290 developed local
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Table 1. Association of miRNA-1290 expression with clinical parameters in sacral chordoma

<table>
<thead>
<tr>
<th>Parameters</th>
<th>miRNA-1290 expression</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>High (n=10)</td>
<td>Low (n=18)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
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</tr>
<tr>
<td>&lt;50</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>≥50</td>
<td>5</td>
<td>14</td>
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<td></td>
</tr>
<tr>
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<td>14</td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Tumor location</td>
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<tr>
<td>Above S3</td>
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<td>12</td>
</tr>
<tr>
<td>S3 and below</td>
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</tr>
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</tr>
<tr>
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</tr>
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<td>4</td>
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<tr>
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</tr>
<tr>
<td>No</td>
<td>8</td>
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</tr>
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</table>

*P<0.05.

Discussion

Sacral chordoma is a rare bone neoplasm characterized by strong invasiveness and high probability of recurrence [17]. The tumor is resistant to chemotherapy; consequently, surgery is the mainly recommended therapy [11]. However, despite the huge challenge for surgeons to perform complete resection, the mass still tends to recur even operates an en bloc resection [18]. This provokes us to discover relevant biomarkers associated with the clinical features of chordoma. Recently, miRNA has been well studied and there are countless inspiring findings that a variety of miRNAs may play an important role in tumor pathophysiology, including invasion, recurrence, proliferation, metastasis [19-21]. Our previous study has demonstrated the different expression of miRNAs between chordoma and nucleus pulposus with miRNA microarray profiling and revealed that miRNA-1290 is the most down-regulated miRNA within the chromosome 1 [16]. It is worth mentioning that Chromosome 1 gene deletion or amplification is the most common chromosome variation in chordoma [22-24]. In this study, the different expression of miRNA-1290 was confirmed and clinical features as well as prognosis of chordoma were analyzed.

Chordoma shares favorable homology with fetal nucleus pulposus due to the fact that chordoma originates from notochord remnants, which shows great advantages over adjacent normal tissues. We detected the expression of miRNA-1290 in chordoma tissues and nucleus pulposus tissues. The result demonstrated that the expression of miRNA-1290 in chordoma was significantly lower than in fetal nucleus pulposus (P=0.026). Consistent with our result, Endo et al. reported that miRNA-1290 was down-regulated in ER^{high} Ki67^{low} breast cancer cohorts [25]. There were also findings reporting that miRNA-1290 was significantly varied in some sort of tumors [26, 27]. All the above indicate that miRNA-1290 plays a crucial role in the molecular mechanism of the occurrence and development of chordoma.

Recently, many researchers have revealed that miRNAs function as pivotal regulators in chordoma cellular events. Duan et al. found that restoration of miR-1 expression suppresses migratory and invasive activities of chordoma cells [28] and verified that by further experiment showing that miR-1 reduces cell population growth and proliferation in chordoma cells in vitro [29]. Eiji Osaka’ team reported that miR-1 reduces cell proliferation, as well as migratory and invasive activities of chordoma cells [30]. Coincidently, our result suggests that dysregulation of miRNA-1290 significantly correlates with the tumor invasion into surrounding tissues (P=0.013), rather than other clinical features ranging from age, gender, tumor size to tumor location. What’s more, evidence has shown miRNA-1290 occupies a vital position impacting on tumor cell proliferation and metastasis, reprogramming, even anticancer reaction [27, 31, 32]. Taken together, we deem that abnormally expressed miRNA-1290 may
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be of great value in predicting the aggressiveness and malignancy of chordoma.

As one of the most troublesome features of chordoma, local recurrence is still a remaining problem to be solved [17, 18], and always count against the prognosis of patients. Thus far, there is only one article explored the relationship between miRNA-1290 and tumor recurrence. Within colorectal cancer cohorts, miRNA-1290 was found to have great potential to be a predictive biomarker of tumor recurrence and the Cox's proportional hazard regression model was employed to demonstrate that serum miRNA-1290 could be an independent risk factor for early recurrence [33]. Current study found that differential miRNA-1290 was associated with local recurrence (P=0.03). In addition, Kaplan-Meier survival curve and log-rank test revealed that RFS was significantly shorter in patients with low-level miRNA-1290 (P=0.004). Besides the colorectal cancer, Mo et al. also draw a conclusion that disordered miRNA-1290 was closely related with the prognosis of non-small cell lung cancer patients [26, 33]. Also, similar phenomenon has been observed in other miRNAs involved in chordoma. Duan et al. reported that miRNA-1 expres- 
sion levels were related to the outcome clinical prognosis for patients [29]. The miR-140-3p and miR-1237-3p were reported to have strong relationships with the prognosis and were identified as an independent predictor for short recurrence-free survival of spinal chordoma patients [34, 35]. As a result, miRNA-1290 may be proved to become a crucial prognostic indicator for sacral chordoma.

Notably, some articles concluded that the miRNA-1290 expression was up-regulated in tumor microenvironment while our result showed the opposite change [26, 36]. Still, consensuses are made that deregulated miRNA-1290 expression could promote tumor invasion and recurrence and significantly associated with the prognosis, which indicates that miRNA-1290 may function as not only oncogene but also tumor suppressor to alter tumor characteristics. Supported by discovery proposing that down-regulated miRNA-1290 was linked with preferable subtype of the breast cancer [25], further studies are welldeserved to clarify the molecular mechanism of miRNA-1290 in chordoma genesis and progression.

In conclusion, examined by quantitative real-time PCR, low expression of miRNA-1290 was found within sacral chordoma tissues, and was significantly correlated with the invasion and recurrence of chordoma. Our result suggests that miRNA-1290 might become a valuable biomarker in predicting prognosis within sacral chordoma patients. Furthermore, it might serve as an effective therapeutic target for sacral chordoma in the near future.

Acknowledgements

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Disclosure of conflict of interest

None.

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References


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