Peroxiredoxin 1 (PRDX1) Suppresses Progressions and Metastasis of Osteosarcoma and Fibrosarcoma of Bone

Corresponding Author: Hao Peng, e-mail: penghaowuda@163.com

Authors’ Contribution: Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

Backgrounds: Osteosarcoma and fibrosarcoma are malignant tumors with poor prognosis. Peroxiredoxin 1 (PRDX1) is considered to prevent tumors in many malignances. However, few studies have focused on the functions of PRDX1 in osteosarcoma and fibrosarcoma.

Material/Methods: PRDX1 mRNA in tumors and adjacent tissues of 32 osteosarcoma patients and 16 fibrosarcoma patients was extracted and measured. Proliferation and invasion of MG63 and HT1080 cell lines after silencing or overexpressing PRDX1 were used to detect the role of PRDX1 in metastasis of osteosarcoma and fibrosarcoma.

Results: PRDX1 mRNA level was lower in tumor tissues than in adjacent tissues of osteosarcoma (F=50.105) and fibrosarcoma (F=28.472) patients, both significantly (P<0.05). Silencing PRDX1 promoted proliferation of MG63 and HT1080 cells, while overexpressing PRDX1 suppressed proliferation after 24 h, 48 h, and 72 h, compared to the control group, both significantly (P<0.05). Silencing PRDX1 increased invasive cells of MG63 (F=246.218) and HT1080 (F=245.602), while overexpressing PRDX1 decreased invasive cells of both, compared to the control, and the difference was significant (P<0.05).

Conclusions: PRDX1 expression is low in osteosarcoma and fibrosarcoma tumors. PRDX1 suppressed the progression and metastasis of osteosarcoma and fibrosarcoma cells.

MeSH Keywords: Cell Proliferation • Fibrosarcoma • Neoplasm Invasiveness • Osteosarcoma • Peroxiredoxins

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/908736
Osteosarcoma is the most common primary bone carcinoma occurring in children and adolescents, with high mortality and poor prognosis around the world [1]. According to some studies, osteosarcoma incidence is higher in males than in females [2,3]. In 1990–2004, boys had significantly higher mortality than girls in the United States [4]. Over the past few years, the main treatment strategy for osteosarcoma is preoperative chemotherapy followed by surgery, and adjuvant therapy greatly improves the survival rate [5,6]. However, tumor stage, metastasis, recurrence, chemotherapeutic treatment, and tumor location, size, and percentage of tumor cells destroyed after neoadjuvant chemotherapy are considered to have important effects on survival rates of osteosarcoma patients [7,8].

Fibrosarcoma of bone is a primarily malignant mesenchymal tumor, which is derived from fibrous connective tissue [9]. Fibrosarcoma originates in fibrous tissues of the bone and invades long or flat bones [10]. Only about 5% of all primary bone sarcomas are fibrosarcoma [11]. Fibrosarcoma usually has historical features similar to those found in other bone tumors, making fibrosarcoma indistinguishable from other bone tumors. Treatment of fibrosarcoma is similar that of other sarcomas of bone. As the mainstay for controlling the local disease, surgical extirpation is the primary strategy, but has poor prognosis [12–14].

Reactive oxygen species (ROS) are free radicals that are involved in many cellular metabolism and signalling pathways, and are thought to play roles in carcinogenesis and ageing [15,16]. Peroxiredoxins (PRDXs) are small H₂O₂ scavenging proteins, with tumor-preventive functions, especially PRDX1 and PRDX2 [17]. Many researchers have confirmed the role of PRDX1 as a tumor suppressor by gene knockout experiments in mice [18,19]. Although PRDX1 has been studied extensively in breast cancer [20], lung cancer [21], and prostate cancer [22], the function and regulation of different PRDXs in various tumors are still poorly understood. PRDXs protect cells from ROS insults and also regulate transduction pathways, utilizing c-Abl, caspases, nuclear factor-κB (NF-κB), and activator protein-1 (AP-1) to influence cell growth and apoptosis [23–25]. Specific drugs targeting PRDXs in cancers are now receiving much research attention as adjuvant therapy in treating cancers.

Few studies have focused on the role of PRDXs in osteosarcoma or fibrosarcoma of bone, and the molecular mechanisms of PRDXs in osteosarcoma and fibrosarcoma need further investigations. In the present study, we used 2 cell lines – MG63 for osteosarcoma and HT1080 for fibrosarcoma – in the cell experiments. Proliferation and invasion were measured after silencing or overexpressing PRDX1, in order to confirm the effect of PRDX1 on progression of osteosarcoma and fibrosarcoma.

Material and Methods

Patients

We enrolled 32 patients with osteosarcoma and 16 with fibrosarcoma. All patients were diagnosed and received surgical resection without additional treatments in Renmin Hospital of Wuhan University from 1 June 2015 to 31 December 2016. Informed consent was obtained from each patient, and the Medical Ethics Committee of Renmin Hospital of Wuhan University approved the study.

Tumor RNA extraction and PCR

Tumor and adjacent tissues were obtained by surgery and ground with liquid nitrogen. We treated 50–100-mg tissue samples with 1 ml TRIzol reagent (Invitrogen, Thermo Fisher Scientific, Inc., Shanghai, China) and a homogenate. RNA was extracted and purified with an RNA Purification Kit (Invitrogen, Thermo) according to the specifications. Primers of PRDX1 (forward: 5’-CAGCCCCAGCCCTCACTTCGGC-3’, reverse: 5’-CAGACCCGAAAGCACCATTGC-3’) and GAPDH (forward: 5’-GAAGGGTGAGTGGGATTTC-3’, reverse: 5’-GAAGATCGTGATGGGATTTC-3’) were synthesized by Invitrogen (Thermo). SuperScript™ IV One-Step RT-PCR System (Invitrogen, Thermo) was used for PCR. A total of 45 cycles of 40 s at 94°C for 1 min at 65°C and 90 s at 72°C were performed. Results were analyzed by LightCycler96 (Hoffmann-La Roche Ltd. Shanghai, China).

Western blot

Proteins were extracted from tissues using RIPA Lysis and Extraction Buffer (Thermo) according to the manufacturer’s specifications. Proteins were quantified by BCA protein assay kit (Beyotime Biotechnology Company, Shanghai, China). We analyzed 40-μg protein samples by 10% separating gel with 5% stacking gel, transferred to polyvinylidene fluoride (PVDF, Merck Millipore Corporation. Darmstadt, Germany), blocked with 5% non-fat milk in phosphate buffer solution (PBS, Boster Biological Technology Co., Ltd. Wuhan, China) containing 0.05% Tween-20 (Sigma-Aldrich Inc. Shanghai, China), and then separately incubated with anti-PRDX1 (1: 1000, Abcam plc. Shanghai, China) and β-actin (1: 8000, Abcam) antibodies at 4°C overnight. We incubated the protein bands with goat anti-rabbit secondary antibody (1: 3000, Jackson ImmunoResearch Inc. West Grove, PA, USA) at 25°C for 1 h, and then measured the proteins with electrochemiluminescence (ECL, Millipore).

Cells culture

MG63 and HT1080 cell lines were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA). MG63 was cultured in Dulbecco’s modified Eagle’s medium (DMEM,
Gibco™, Thermo). HT1080 was cultured in Minimum Essential Medium (MEM) with Non-Essential Amino Acids (NEAA) (Gibco™, Thermo). The entire medium contained 10% fetal bovine serum (FBS, Gibco™, Thermo), 100 U/ml of penicillin (Sigma) and 100 μg/ml of streptomycin (Sigma). Cells were placed in a CO₂ incubator (Series 8000 Water-Jacketed CO₂ Incubators, Thermo) maintained at 37°C, 5% CO₂, and saturates humidity. At 90% confluence, cells were digested with 0.25% trypsin-EDTA (Gibco™, Thermo) for subculturing.

Silencing PRDX1

RNA interfering (RNAi) assay was used for silencing PRDX1. Small interfering RNA (siRNA) targeting PRDX1 (sc-36177) was purchased from Santa Cruz Biotechnology (Shanghai, China) and resuspended in 330 μl of RNAse-free water (Santa), with a primary concentration of 10 μM. Cells were starved for 6 h in medium without FBS. RPDX1 siRNA was transfected into cells with Lipofectamine™ 3000 transfection reagents (Invitrogen, Thermo) according to the manufacturer’s specifications.

Overexpressing PRDX1

pFRT/TO/HIS/FLAG/HA-RPDX1 plasmid (#38086) was purchased from Addgene (Beijing Zhongyuan, Ltd, China). DH5α with plasmid was cultured with LB Broth (Invitrogen, Thermo) containing 100 μg/ml of ampicillin (Sigma) at 37°C for 8–12 h. Plasmid was extracted with the Plasmid Mini Preparation Kit (Beyotime), and then transfected into cells with Lipofectamine™ 3000 transfection reagents (Invitrogen, Thermo) according to the manufacturer’s specifications.

Cellular PRDX1 detection

Cellular proteins were extracted and Western blot analysis was carried out according to the assay mentioned before.

Proliferation detection

Cells were adjusted as 400 cells per well and cultured in a 96-well plate (Corning Inc.) for 24 h. We added 10 μl of PrestoBlue™ Cell Viability Reagent (Thermo) to each well, followed by incubation at 37°C for 10 min. Absorbance was measured at 570 nm (600 nm as reference wave length) with a microplate reader (Multiskan™ FC, Thermo). Optical density (OD) values were evaluated for 3 consecutive days.

Invasion detection

Matrigel (Becton, Dickinson, and Co., Shanghai, China) was diluted in medium (5%). A Transwell Insert of Transwell permeable supports (Corning) was covered with diluted Matrigel and incubated at 37°C for 4 h. Cells were starved for 6 h and then adjusted as 1×10⁵ cells/ml in medium without FBS. Cells were then seeded in the Transwell insert. The entire medium with 10% FBS was added into the lower Transwell insert. Culturing was continued for 12 h, then we washed the cells with PBS. We fixed the cells with methanol (Solarbio Sciences and Technology Co., Ltd., Beijing, China) for 30 min and then stained them with 0.4% crystal violet (Solarbio) in 20% methanol for 20 min. Non-transmembrane cells were cleaned. Cells were observed under microscopy (Eclipse Ts2, Nikon Instrument, Inc. Japan). We randomly selected 5 fields and calculated the number of transmembrane cells.

Statistical analysis

Data were analyzed by SPSS 21.0 software and are shown as mean±standard deviation (x±SD). One-Way ANOVA was used for analyzing comparisons between groups. LSD was used for homogeneity of variance, while Dunnett’s T3 was used for heterogeneity of variance. A value of P<0.05 was considered significant.

Results

Lower PRDX1 mRNA was found in tumor tissues

PRDX1 mRNA levels in tumor and adjacent tissues are shown in Table 1 and Figure 1. For patients with osteosarcoma, PRDX1 mRNA levels in tumor tissue (14.45±10.21) IU/mL were significantly (F=50.105, P<0.001) lower than in the adjacent tissue.
In patients with fibrosarcoma, PRDX1 mRNA levels in tumor tissue (18.51±8.73) IU/mL were significantly lower than in the adjacent tissue (141.73±91.96) IU/mL (F=28.472, P<0.001). These results indicate that PRDX1 expression is low in osteosarcoma and fibrosarcoma tumor tissues.

PRDX1 suppressed tumor cells proliferation

Results of detecting MG63 and HT1080 proliferation are shown in Table 2 and Figures 2 and 3. Figure 2 shows the successful silencing or overexpressing of PRDX1 in MG63 and HT1080 cell lines.

As shown in Figure 3, in all 3 groups, MG63 and HT1080 cells both grew over time, with significantly increased OD values (P<0.001).

Silencing PRDX1 significantly promoted the proliferation of MG63 cell after 24 h (F=11.718, P=0.027), 48 h (F=98.538, P=0.001), and 72 h (F=40.534, P=0.003), compared to the control, and silencing PRDX1 significantly promoted the proliferation of HT1080 cell after 24 h (F=75.625, P=0.001), 48 h (F=169.356, P<0.001) and 72 h (F=29.509, P=0.006) compared to control. However, overexpressing PRDX1 significantly decreased the proliferation of MG63 at 24 h (F=150.224, P<0.001), 48 h (F=126.499, P<0.001), and 72 h (F=168.894, P<0.001), compared to the control. Overexpressing PRDX1 also significantly reduced the proliferation of HT1080 at 24 h (F=62.253, P=0.001), 48 h (F=186.397, P<0.001), and 72h (F=87.604, P=0.001), compared to control.

These results indicate that PRDX1 suppressed the proliferation of MG63 and HT1080 cells, suggesting the inhibition of progression of osteosarcoma and fibrosarcoma.
PRDX1 suppressed tumor cell invasion

Results of detecting MG63 and HT1080 invasion are shown in Table 3 and Figure 3. Silencing PRDX1 increased the invasive-ness of MG63 ($F_{24h}=228.742$, $P<0.001$) and HT1080 ($F_{24h}=174.724$, $P<0.001$) cells, while overexpressing PRDX1 significantly de creased the invasiveness MG63 ($F_{24h}=29.538$, $P=0.006$) and HT1080 ($F_{24h}=89.471$, $P=0.001$) cells. The above results indicate that PRDX1 suppressed MG63 and HT1080 invasion, suggesting the inhibition of metastasis of osteosarcoma and fibrosarcoma.

Discussion

We collected the tumor and adjacent tissues of patients with osteosarcoma or fibrosarcoma, and then measured PRDX1 mRNA levels. We first found that PRDX1 mRNA level was significantly ($P<0.05$) lower in the tumor tissues than in the adjacent tissue, not only in osteosarcoma but also in fibrosar coma. Secondly, we used RNAi and plasmid transfection as says to detect the effects of PRDX1 on the proliferative ability and invasive ability of MG63 and HT1080 cell lines. We found that silencing PRDX1 promoted the proliferative ability and invasive ability of both MG63 and HT1080 cells, while over expressing PRDX1 suppressed the proliferative ability and invasive ability of both MG63 and HT1080 cells. These results indicate that PRDX1 acts as an inhibitor of progression and metastasis of osteosarcoma and fibrosarcoma.

Osteosarcoma is a type of malignant tumor occurring in bone and the most commonly histological form of primary bone cancer [26], mostly being prevalent in teenage and young adults [27]. Poor prognosis of osteosarcoma is mainly for the metastasis, usually to lung, limb bones and so on. Resection

Table 2. PRDX1 suppressing MG63 and HT1080 cells proliferation (OD value, $\pm SD$).

|          | MG63            |          | HT1080           |          |
|----------|-----------------|----------|------------------|----------|
|          | 24 h | 48 h | 72 h | $F$ value | $P$ value | 24 h | 48 h | 72 h | $F$ value | $P$ value |
| Con.     | 0.26± 0.02 | 0.60± 0.02 | 0.93± 0.05 | 293.810 | <0.001 | 0.19± 0.01 | 0.58± 0.03 | 0.86± 0.06 | 200.273 | <0.001 |
| siRNA    | 0.34± 0.03 | 0.83± 0.03* | 1.13± 0.02** | 565.648 | <0.001 | 0.33± 0.02 | 0.92± 0.03* | 1.07± 0.03** | 547.842 | <0.001 |
| Over.    | 0.14± 0.06 | 0.35± 0.03* | 0.51± 0.03** | 53.086 | <0.001 | 0.11± 0.01 | 0.30± 0.01* | 0.51± 0.02** | 572.916 | <0.001 |
| $F$ value | 74.314 | 206.334 | 27.214 | <0.001 | 127.754 | 372.876 | 145.453 | <0.001 | <0.001 |
| $P$ value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

PRDX1 – peroxiredoxin 1; OD – optical density; Con. – control group; siRNA – silencing PRDX1; Over. – overexpressing PRDX1; * comparing to Con., $P<0.05$; # comparing to siRNA, $P<0.05$.
is the initial treatment, but it could not prevent osteosarcoma metastasis. Fibrosarcoma is also a malignant tumor, which is derived from fibrous connective tissue. Fibrosarcoma was usually found in males with poor prognosis for metastasis, especially to breast [28–31]. Chemotherapy or radiotherapy has poor effects on treating fibrosarcoma, and surgical resection or amputation is considered as the primary treatment.

Until now, many genes have been found to be the biomarkers of osteosarcoma, such as SGMS2, SOD1, SPOPL and so on [32]. Genetic polymorphisms are also associated with osteosarcoma progression [33]. According to Zheng et al., IGF-1 is one of the targets in leptocarpin suppressing osteosarcoma progression [34]. However, no research reports the role of PRDX1 in osteosarcoma, even in fibrosarcoma.

PRDX1 is a factor originating from human red blood cells. Most research on PRDX1 is based on cell lacking PRDX1 or murine isoforms with PRDX1 knockout, and the results suggest PRDX1 is a tumor suppressor [18,35]. The role of PRDX1 in tumor

Figure 3. PRDX1 suppressed MG63 and HT1080 cells invasion (PRDX1 – peroxiredoxin 1; Con. – control group; siRNA – silencing PRDX1; Over. – overexpressing PRDX1; * compared to Con., P<0.05; # compared to siRNA, P<0.05).
Table 3. PRDX1 suppressing MG63 and HT1080 cells invasion (invasive cell, \(\chi \pm SD\)).

|           | MG63      | HT1080    |
|-----------|-----------|-----------|
| Con.      | 29.00±3.61| 36.33±4.04|
| siRNA     | 115.00±9.17* | 106.00±8.16* |
| Over.     | 13.00±3.61** | 10.33±2.52** |
| F value   | 246.218   | 245.602   |
| P value   | <0.001    | <0.001    |

PRDX1 – peroxiredoxin 1; Con. – control group; siRNA – silencing PRDX1; Over. – overexpressing PRDX1; * comparing to Con., \(P<0.05\); ** comparing to siRNA, \(P<0.05\).

We used the RNAi approach to silence PRDX1 in MG63 and HT1080 cell lines. On one hand, by measuring the proliferation with CCK-8, we confirmed that silencing PRDX1 promoted the proliferation of MG63 and HT1080 cell lines, significantly. We also showed that transfecting plasmids with overexpressing PRDX1 suppressed the growth of MG63 and HT1080 cell lines. One the other hand, the invasive ability of MG63 and HT1080 cell lines were also measured after silencing or overexpressing PRDX1. The results showed that silencing PRDX1 promoted the invasive ability of both MG63 and HT1080 cell lines, while overexpressing PRDX1 suppressed the invasive ability of both cell lines. All of these results indicate the inhibition of PRDX1 on the growth-arresting process and metastasis of MG63 and HT1080 cell lines, which suggests that PRDX1 also acts as a tumor suppressor in the development and metastasis of osteosarcoma and fibrosarcoma.

Due to time constraints, we did not collect enough clinical data on the osteosarcoma and fibrosarcoma patients, especially the patient prognosis. The correlation between PRDX1 with metastasis of osteosarcoma and fibrosarcoma in patients after receiving surgical resection remains unclear. Another limit is that we did not establish a mouse model lacking PRDX1. The role of PRDX1 in osteosarcoma and fibrosarcoma has not been further confirmed in vivo. In further experiments, we will first follow the patients after surgery for more than 5 years and analyze the correlation between PRDX1 level in tumor tissues with metastasis of osteosarcoma and fibrosarcoma. Secondly, we will establish PRDX1-deficient mice or inject mice with MG63 or HT1080 cell lines with silencing PRDX1, in order to confirm the importance of PRDX1 in regulating metastasis of osteosarcoma and fibrosarcoma. Finally, signal pathways related to PRDX1, especially those related to ROS, should be further investigated.

Conclusions

This is the first study showing inhibition of PRDX1 in the development and metastasis of osteosarcoma and fibrosarcoma, which provides clinical research on PRDX1 in progression of osteosarcoma and fibrosarcoma, suggesting the molecular mechanisms and potential treatment targets.

Conflict of interest

None.
1. Dorfman HD, Czerniak B: Bone cancers. Cancer, 2015; 75(Suppl. 1): 203–10
2. Ries LAG, Smith MA, Gurney JG et al: Cancer incidence and survival among children and adolescents: United States SEER Program, 1973–1995. 1999
3. Dahlin DC, Unni KK: Bone tumors: General aspects and data on 8,547 cases. 4th ed. Basic Biologicales, 1986
4. State-specific influenza vaccination coverage among adults aged >18 years — United States, 2003–04 and 2005–06 influenza seasons. MMWR Morb Mortal Wkly Rep, 2008; 57(38): 1033
5. Link MP, Goorin AM, Miser AW et al: The effect of adjuvant chemotherapy on relapse-free survival in patients with osteosarcoma of the extremity. N Engl J Med, 1986; 314(25): 1600–6
6. Meyers PA, Schwartz CL, Kralio MD et al: Osteosarcoma: the addition of murrayal tripeptide to chemotherapy improves overall survival — a report from the Children’s Oncology Group. J Clin Oncol, 2008; 26(4): 633–38
7. Jaffe N: Progress in pediatric hematology/oncology, Vol 2: Bone Tumors In Children, 1979
8. Mankin HJ, Hornicek FJ, Rosenberg AE et al: Survival data for 648 patients with osteosarcoma treated at one institution. Clin Orthop Relat Res, 2004; 429(429): 286–91
9. Cimmino-3Mathews A, Harvey SC, Argani P: Benign and malignant mesenchymal tumors. Springer International Publishing, 2016
10. Edwards TM, Duran MS, Meeker TM: Congenital infantile fibrosarcoma in the premature infant. Adv Neonatal Care, 2017; 17(6): 440–50
11. Dorfman HD, Czerniak B: Bone cancers. Cancer, 1995; 75(1 Suppl.): 203–10
12. Papagelopoulos PJ, Galanis E, Frassica FJ et al: Primary fibrosarcoma of bone: outcome after primary surgical treatment. Clin Orthop Relat Res, 2000; 373(373): 88–103
13. Kanno A, Hatori M, Hosaka M et al: Multiple bone metastasis of sclerosing epitheloid fibrosarcoma 12 years after initial surgery-increasing ki-67 labeling index. Sarcoma, 2009; 2009: 953750
14. Landra AP, Ruberti RF: Recurrent fibrosarcoma of skull: A 35-year surgical odyssey. Br J Plastic Surg, 1981; 34(4): 462–63
15. Nicolussi A, D’Inzeo S, Mincione G et al: PRDX1 and PRDX6 are repressed in epithelioid fibrosarcoma 12 years after initial surgery-increasing ki-67 labeling index. Sarcoma, 2009; 2009: 953750
16. Shi Y, Yang F, Wei S, Xu G: Identification of key genes affecting results of hyperthermia in osteosarcoma based on integrative ChIP-Seq/TargetScan analysis. Med Sci Monit, 2017; 23: 2042–48
17. Mao J, Zhuang G, Chen Z: Genetic polymorphisms of insulin-like growth factor 1 are associated with osteosarcoma risk and prognosis. Med Sci Monit, 2017; 23: 5892–98
18. Lo Muzio L, Mignogna MD, Pannone G: A rare case of fibrosarcoma of the jaws in a 4-year-old male. Oral Oncol, 1998; 34(5): 383–86
19. Xiong C, Chen YH, Zhang Y et al: Magnetic resonance imaging and mammographic appearance of dermamtrifosarcoma protubers in a male breast: a case report and literature review. J Med Case Rep, 2009; 3: 8246
20. Akhtar K, Sherwani R, Sen PR: Dermatofibrosarcoma protuberans of male breast: An unusual presentation. Oman Med J, 2012; 27(4): e038
21. Shukla S, Chauhan R, Iyotsna PL, Andley M: Primary fibrosarcoma of male breast: A rare entity. J Clin Diagn Res, 2014; 8(4); FD11–12
22. Basu A, Banerjee H, Rojas H et al: Differential expression of peroxiredoxins in prostate cancer: Consistent upregulation of PRDX3 and PRDX4. Prostate, 2011; 71(7): 755–65
23. Wang Y. et al.: PRDX1 suppresses progression of lung cancer through epithelial-mesenchymal transition. Journal of Zunyi Medical University, 2015 [in Chinese]
24. Dongyun XU, Xiaojing HE, Wang J et al: Expression of Prdx1 in gastric cancer and its clinical significance. Chinese Clinical Oncology, 2014 [in Chinese]