Overexpression of Polo-like kinase1 (PLK1) in chondrosarcoma and its implications for cancer progression

Kelu Li1,2*, Hongmei Ma2*, Xiuyan Zheng2, Yali Hu2, Yue Wang2, Kunpeng Zhang2, Jiahan Chen3, Yan Qi1,2, Jinfang Jiang1,2, Lijuan Pang1,2, Lin Tao1,2, Wenyi Gu4, Feng Li5, Hong Zou1,2

1Department of Pathology, First Affiliated Hospital, Shihezi University, Shihezi, China; 2Key Laboratory of Xinjiang Endemic and Ethnic Diseases, Department of Pathology, School of Medicine, Shihezi University, Ministry of Education of China, Shihezi, China; 3School of Medicine, Shihezi University, Shihezi, China; 4Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, Australia; 5Department of Pathology, Beijing Chaoyang Hospital, Capital Medical University, Beijing, China. *Equal contributors.

Received April 22, 2017; Accepted July 5, 2017; Epub March 1, 2018; Published March 15, 2018

Abstract: Polo-like kinase1 (PLK1) is a new therapeutic target for osteosarcoma with good application prospects. Whether PLK1 is highly expressed in chondrosarcoma and whether PLK1 can be a potential therapeutic target for chondrosarcoma are worth exploring. However, PLK1 expression in chondrosarcoma is scarcely investigated. Therefore, we collected 11 cases of chondrosarcoma and 26 cases of osteochondroma with complete clinical pathological data and used immunohistochemical staining to detect the expression of PLK1 in chondrosarcoma and osteochondroma and then studied its significance and relationship with clinical pathological parameters. Our results showed that the positive expression rate of PLK1 in chondrosarcoma tissue (90.91%, 10/11) was significantly higher than the rate of osteochondroma tissues (53.85%, 14/26) (P<0.05). The expression of PLK1 enhanced gradually with the increase in histological grade (P<0.05). PLK1 was highly expressed in chondrosarcoma, and the high expression of PLK1 might be involved in cartilage tumor malignant progression.

Keywords: PLK1, chondrosarcoma, osteochondroma, immunohistochemistry

Introduction

Chondrosarcoma is a highly malignant tumor and accounts for 10%-20% [1] of primary malignant bone tumors. The tumor has the capability of local infiltration and metastasis and is insensitive to radiation or chemotherapy. Complete surgical resection is still the main therapy for chondrosarcoma. Most patients’ prognosis is poor because of the lack of effective auxiliary treatment [2]. Thus, new therapeutic targets must be discovered. Polo-like kinase1 (PLK1) is a member of the family of PLKs and widely exists in eukaryotic of silk/threonine protein kinase. Given that PLK1 plays an important role in cell cycle, this gene has attracted much attention in recent years. PLK1 is highly expressed in most tumors, such as osteosarcoma [3], non-small-cell lung cancer (NSCLC) [4], and breast cancer [5], and is closely related to the tumor cell proliferation and prognosis of patients. In 2013, Wenyi Gu [6] found that PLK1 is highly expressed in osteosarcoma cell lines (KHOS), and the growth of osteosarcoma can be significantly inhibited using nanometer material carrier SiRNA technology to silence PLK1 gene. Thereafter, Chou YS [7] found that PLK1 inhibitor gsk461364 can terminate osteosarcoma cell mitosis, inhibit tumorous cellular growths, and accelerate cell apoptosis. Therefore, PLK1 is considered a good application prospect of new therapeutic target for osteosarcoma. No related studies on PLK1 expression in chondrosarcoma are available, and whether PLK1 can be potential therapeutic targets of chondrosarcoma is still unknown. As a result, these issues must be explored. Hence, we used the immunohistochemical method to detect the expression of PLK1 in chondrosarcoma, contrasted the said expression to the
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PLK1 expression of osteochondroma to explore its relationship with clinicopathological parameters and meaning, and laid the foundation for further discussion of the possible clinical application.

Materials and methods

Reagents

Rabbit anti-human PLK1 monoclonal antibody (ab15529, clone number #2602) was purchased from Abcam of England. Envision staining kits, PBS buffer powder, and two resistance and DAB chromogenic reagents used for immunohistochemistry were purchased from Zhongshan Jinqiao Biological Technology Co., Ltd. (Beijing, China).

Patients and tissue specimens

A total of 11 cases of chondrosarcoma and 26 cases of osteochondroma were collected from the first affiliated hospital of Shihezi University School of Medicine from 2009 to 2016. All the patients had complete medical historical and clinical pathological data. All the cases were confirmed by operation and pathology. Paraffin block and corresponding HE sections were collected, and HE sections were read by two senior pathologists. Chondrosarcomas were grouped and classified according to the WHO standards.

Immunohistochemical staining

The two-step immunohistochemical envision method was applied. The cases of chondrosarcoma and osteochondroma organization were used as samples of 5 μm-thick serial section. Samples were dried, followed by conventional xylene dewaxing, hydration, gradient alcohol at 20% EDTA antigen repairing buffer (PH 9.0) in high temperature and high pressure antigen for 8 min. Then, samples underwent natural cooling to room temperature, 3% H₂O₂ incubation for 10 min at room temperature, and removal of endogenous hydrogen peroxide enzyme. The rabbit anti-monoclonal antibody was added (1:200 dilution), followed by 4°C incubation overnight. The rabbit mouse universal biotin

Figure 1. A and B: Show the low- and high-power microscopic images of a chondrosarcoma; C: Shows the strongly positive expression of PLK1 in the tissue of chondrosarcoma (3+) and its detected location in the cell nucleus. D and E: Show the low- and high-power microscopic images of osteochondroma; F: Shows the negative or weakly positive expression of PLK1 in the tissue of osteochondroma (0-1+) and its detected location in the cell nucleus.
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HuaEr resistance and hydrogen peroxide content were used to label the antibodies. Then, DAB chromogenic, hematoxylin redyeing was conducted for 2 min, followed by dehydration of transparent seal. With known positive colon tissue slices as positive control, PBS solution was used instead of a resistance as a blank control.

Result processing

The positive expression of PLK1 was located in the cell nucleus and presented a dark-brown granulation. Each slice was then selected using five high-power microscopes (400×) for image acquisition. The results were determined on the basis of the percentage of positive cells and the depth of positive staining. The scoring criteria were as follows: IHC staining slides were scored as positive or negative by percentage and intensity of positive cells. The scoring percentages of positively stained cells were as follows: 0≤5%, 1 = 6%-25%, 2 = 26%-50%, 3 = 51%-75%, and 4 = 76%-100%. The scoring of staining intensity was as follows: 0 = absent, 1 = weak, 2 = moderate, and 3 = strong. The final score was based on multiplying both scores from individual slides, where 0-1 was negative (-), 2-3 was weakly positive (1+), 4-6 was moderately positive (2+), and 8-12 was strongly positive (3+).

Statistical analysis

The positive expression of PLK1 between chondrosarcoma and osteochondroma tissues were compared by X² test. Kruskal-Wallis rank test was applied to analyze correlation between expression of PLK1 and clinicopathological parameters in chondrosarcoma. All analysis was performed by SPSS version 17.0, and p-values <0.05 were considered to be significant.

Results

PLK1 was highly expressed in chondrosarcoma but lowly expressed in osteochondroma (Figure 1; Table 1)

The immunohistochemical staining results showed that PLK1 protein was located in the nucleus. The positive expression rate of chondrosarcoma was 90.91% (9/11), and the majority showed moderately to strong positively (2-3+). The positive expression rate of osteochondroma was 53.85% (14/26), and all the 14 cases were weakly positive (1+). A significant difference in PLK1 expression between chondrosarcoma and osteochondroma was found (P = 0.014, Table 1). Among the 14 cases of positively expressed osteochondroma, 57.14% (8/14) were multiple osteochondroma. PLK1 was negatively expressed in normal bone and cartilage tissue (4/4).

The expression of PLK1 in chondrosarcoma was positively correlated with clinical category (Figure 1; Table 1)

For this group of chondrosarcoma, 6 cases were in grade 1, 2 cases were in grade 2, 3 cases were in grade 3. Among them, PLK1 expression was different between the hierarchical groups: the positive expression rates in levels 1, 2, and 3 were 66.67% (4/6), 100% (2/2), and 100% (3/3) respectively. PLK1 protein was associated with the clinical classification of chondrosarcoma, and PLK1 was negatively expressed in normal cartilage tissue adjacent to tumor tissue of chondrosarcoma. The expression rate of PLK1 in high-grade chondrosarcoma was higher than that in low-grade chondrosarcoma, and the difference was statistically significant (P = 0.017, Table 2).

Discussion

In 1994, Golsteyn [8] was the first to report that PLK1 is located in 16p12. PLK1 is highly expressed in cells with active proliferative abil-
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ity, such as those in the placenta, ovary, testis, and spleen; however, PLK1 does not express or is lowly expressed in most adult organizations (such as the liver, brain, thymus, and heart). PLK1 can regulate the cell cycle, inhibit tumor cell apoptosis, and promote tumor formation. The activity of its kinase is closely related to the development of cell cycle and progress of various types of cancers. Therefore, PLK1 is considered a good application prospect of new therapeutic target for malignant tumor. Preclinical research showed that the interference of PLK1 expression can significantly inhibit the growth of a series of tumor cells, including gastrointestinal [6], nasopharyngeal carcinoma [9], NSCLC [10], lymphoma [11, 12], and colorectal cancer [13]. Interestingly, such interference exerts no obvious effect on normal cells [14]. At present, small molecular PLK1 inhibitors (such as BI2536 and BI6727) or siRNA has entered clinical trials. Duan Z [15] found that using shRNA to silence PLK1 gene of osteosarcoma cell lines KHOS and U-2OS can inhibit cell proliferation and promote apoptosis; their research suggested that PLK1 can be a potential therapeutic target for osteosarcoma. Then, Chou YS [7] verified that PLK1 inhibitor gsk461364 may terminate osteosarcoma cell mitosis, inhibit tumor cell proliferation, and promote cell apoptosis. The findings are consistent with the view that inhibition of PLK1 protein may downregulate cell cycles of osteosarcoma by decreasing the activity of p53, thereby leading to the inhibition of tumor cell proliferation. Given that chondrosarcoma is derived from the bone, whether inhibition of PLK1 can contribute to treatment of tumor is worth exploring. However, PLK1 expression in chondrosarcoma has not been reported to date.

Our study confirmed that PLK1 was highly expressed in chondrosarcoma and was positively related to the clinical classification. The results suggested that high expression of PLK1 might be involved in the malignant progress of chondrosarcoma. This issue needs further study with large sample size. Our research also discovered that PLK1 was negatively expressed or weakly positively expressed in normal bone and cartilage; among these positive expression cases of osteochondroma, 57.14% (8/14) were multiple osteochondroma. Multiple osteochondroma may [16] progress to malignant chondrosarcoma. Whether PLK1 is involved in the malignant progress of cartilage tumor and whether PLK1 is the key event to the malignant transformation of cells deserve further study. Chondrosarcoma is a highly malignant tumor with the capability of local invasion and distant metastasis and is insensitive to radiation or chemotherapy; thus, most patients’ prognosis is poor. PLK1 expression is associated with poor prognosis of a series of tumors, and small-molecule PLK1 inhibitors can increase the sensitivity of chemotherapy drugs in many kinds of tumors, including osteosarcoma [17], pancreatic cancer [18], and liver cancer [19]. Accordingly, the curative effect and the prognosis of patients are enhanced. Whether existing PLK1 inhibitors or other treatments can be effective in chondrosarcoma deserves further research and discussion.

In conclusion, PLK1 protein was highly expressed in chondrosarcoma, and the expression of PLK1 was positively related to the histologic grade of chondrosarcoma. PLK1 might participate in the occurrence and development of chondrosarcoma, and might be associated with malignant progress of cartilage tumor. With further research on PLK1 in cartilage tumor, the antineoplastic drug target for PLK1 gene or protein in combination with commonly used surgery, radiation, and chemotherapy, might be promising cartilage tumor treatment strategies in the near future.

Acknowledgements

This study was supported by grants from the National Natural Science Foundation of China (Nos. 81460383 and 81660411).

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Hong Zou and Lin Tao, Key Laboratory of Xinjiang Endemic and Ethnic Diseases, Department of Pathology, School of Medicine, First Affiliated Hospital, Shihezi University, Ministry of Education of China, Shihezi, China. Tel: +86-13899528366; E-mail: zouhong_patho@163.com (HZ); Tel: +86-13999335086; E-mail: taolin419315@163.com (LT)

References

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