Pathology – Research and Practice 212 (2016) 636–642

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.com/locate/prp

Original article

The clinical pathological significance of Thy1 and CD49f expression in chondrosarcomas

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ARTICLE INFO

Article history:
Received 31 October 2015
Received in revised form 17 March 2016
Accepted 20 April 2016

Keywords:
Thy1
CD49f
Immunohistochemistry
Chondrosarcoma
Osteochondroma

ABSTRACT

Objective: This study investigated the protein expression and clinicopathological significance of Thy1 and CD49f in chondrosarcomas.
Methods: Thy1 and CD49f protein expression in 59 chondrosarcomas and 33 osteochondromas were measured by immunohistochemical staining.
Results: The percentage of positive Thy1 and CD49f expression was significantly higher in patients with chondrosarcoma than in patients with osteochondroma ($P < 0.01$). The percentage of positive Thy1 and CD49f expression was significantly lower in patients with histological grade I, Enneking stage I, AJCC stage I/II stage, non-metastatic and non-invasive chondrosarcoma than in patients with histological grade III, Enneking stage II + III, AJCC stage III/IV, metastatic and invasive chondrosarcoma ($P < 0.05$ or $P < 0.01$). Thy1 expression was positively correlated with CD49f expression in chondrosarcoma. Kaplan-Meier survival analysis showed that histological grade, AJCC stage, Enneking stage, metastasis, invasion, and Thy1 and CD49f expression significantly correlated with shorter mean survival time in chondrosarcoma patients ($P < 0.05$ or $P < 0.01$). Cox multivariate analysis showed that positive Thy1 and CD49f expression was an independent prognostic factor that negatively correlated with overall postoperative survival.
Conclusion: Positive Thy1 and CD49f expression is significantly associated with the progression and poor prognosis of chondrosarcoma.

1. Introduction

Chondrosarcoma is a malignant tumor of the bone accounting for approximately 20% of all malignant bone tumors [1], while osteochondroma is the most common benign tumor of the bone [2]. Chondrosarcoma is derived from cartilage cells and is rarely found in individuals younger than 30 years old. Currently, surgery is the preferred treatment modality for chondrosarcoma because chondrosarcoma is not sensitive to radiotherapy and chemotherapy. Patients with metastatic tumors or tumors located in locations that resection is difficult often have poor prognosis [1]. It is therefore significant to find new treatments. With the development of molecular biology, molecular targeted therapy becomes a hot topic of research. However, molecular therapy of chondrosarcoma is currently unavailable in the clinic due to a lack of specific molecular targets.

Thymocyte antigen 1 (Thy-1) or cluster of differentiation (CD90) is a 25–37 kDa glycosylated membrane protein that is anchored to the cell surface with a single V-like immunoglobulin domain [3]. Thy-1 is found on a variety of cells, including thymocytes and peripheral T cells and is closely associated with the differentiation and recognition of T cells. Thy-1 is also a marker for a variety of stem cells and mature neurons. Thy-1 is also found to be involved in the abnormal proliferation of tumor cells [4]. The current studies on the roles of Thy-1 in malignancies are mainly focused on blood and nervous system cancers [5,6], and only a few studies were conducted on epithelial malignancies [7–9]. However, opposing roles were reported in different cancers. The expression of Thy1 has not been reported in chondrosarcoma. Integrins are a family of important transmembrane glycoprotein receptors composed of the A and B subunits [10], CD49f, also known as integrin A6 (ITGA6), is a cell-surface adhesion molecule and its roles in the tumorigenesis, development, and metastasis of tumors have received increasing attention [11–13]. Recent studies have found that the expression level of CD49f is closely related to the biological behaviors of osteosarcoma [14,15]. However, the expression and significance of CD49f in chondrosarcoma has not been reported.

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http://dx.doi.org/10.1016/j.prp.2016.04.006
0344-0338/

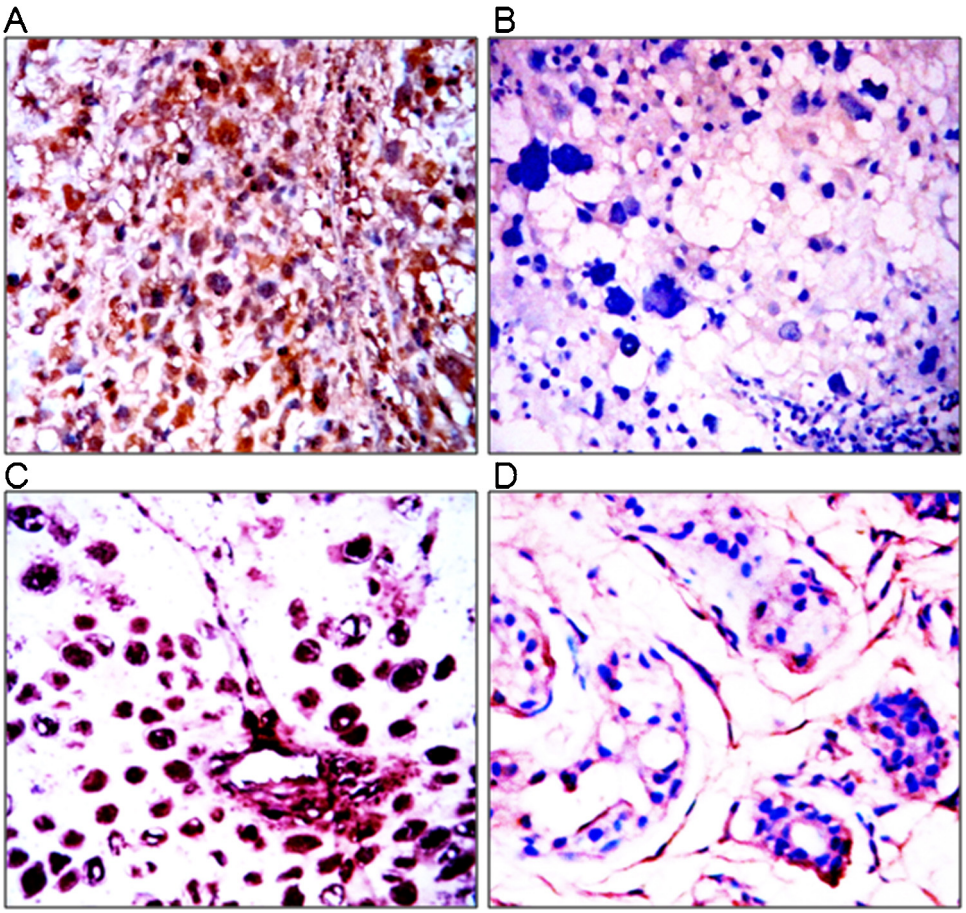

Fig. 1. Immunohistochemical staining of Thy-1 expression. Original magnification x200. Positive reaction was mainly localized in the cytoplasm. A) Positive Thy-1 staining in chondrosarcoma tissues. B) Negative Thy-1 staining in chondrosarcoma tissues. C) Positive Thy-1 staining in osteochondroma tissues. D) Negative Thy-1 staining in osteochondroma tissues.

In this study, the expression of Thy1 and CD49f proteins in chondrosarcoma and osteochondroma was investigated using immunohistochemical methods. The clinical significance of Thy1 and CD49f protein were analyzed.

2. Materials and methods

2.1. Specimens and clinical data

59 resected chondrosarcoma specimens were collected from January 2001 to June 2011 at the Second Xiangya Hospital and Third Xiangya Hospital, Central South University. The diagnosis of chondrosarcoma was confirmed by 2 independent pathologists. Of the 59 patients with chondrosarcoma, 29 were female (49.2%), and 30 were male (50.8%). The pathological types included 53 conventional chondrosarcomas (89.8%), 1 clear cell chondrosarcoma (1.7%), 1 myxoid chondrosarcoma (1.7%), and 4 dedifferentiated chondrosarcomas (6.8%). Of the 59 patients with chondrosarcomas, 24(40.7%), 22(37.3%), and 13(22.0%) patients were histological grade I, IIand III. Of the 59 chondrosarcoma patients, 24 (40.7%), 27 (45.8%), and 8 (13.6%) patients had a maximum tumor diameter $\leq$ 5 cm, 6–10 cm, and >10 cm, respectively. According to the AJCC staging, 10 (16.9%), 34 (57.6%), 5 (8.5%), and 10(16.9%) patients were stage I, II, III and IV, respectively. According to the Enneking staging, 38 (64.4%), 11 (18.6%), and 10 (16.9%) patients were stage I, II, and III, respectively. Metastases were found in 14 (23.7%) patients. Survival information of all chondrosarcoma patients was obtained through phone calls and postal mail with the longest follow-up being 134 months. 13 patients died during the follow-up period (22.0%), while 46 patients survived (censored cases) (78.0%). Thirty three osteochondroma specimens were collected, including 27 from males (81.8%) and 6 from females (18.2%). Of the 33 osteochondroma patients, 29 patients were 45 or younger (87.9%), while 4 patients were older than 45 years old (12.1%). Of the 33 patients, 28 (84.8%) and 5 (15.2%) patients had a maximum tumor diameter $\leq$5 cm and 5 > 5 cm, respectively.

2.2. Immunohistochemistry

Rabbit anti-human Thy1 and CD49f polyclonal antibodies were purchased from Abgent Company (California, CA, USA). Immunohistochemical staining was performed using EnVision™ Detection kit (Dako Laboratories, California, CA, USA) by following the manufacturer's protocol. Briefly, paraffin-embedded tissues were sectioned at 4 μm thick. The sections were deparaffinized and treated with peroxidase inhibitor (3% H_2O_2) in the dark for 15 min after washing with 1xPBS. Slices were incubated with rabbit anti-human Thy1 or CD49f primary antibody for 1 h. After washing with 1 x PBS for 3 × 5 min, solution A was added to the sections for 30 min followed by DAB staining and haematoxylin counter-staining. The slides were then dehydrated with alcohol, soaked in xylene, and mounted with neutral balsam. The positive controls were the positive breast cancer sections provided by Abgent Company. 5% BSA was used in replace of primary antibody as a negative control. 500 cells in 10 random fields were examined per slice and positive Thy1 and CD49f staining at the cytoplasm were accounted as positive