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ORIGINAL ARTICLE

Immunohistochemical analyses of β-catenin and cyclin D1 expression in giant cell tumor of bone (GCTB): A possible role of Wnt pathway in GCTB tumorigenesis

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Abstract

Giant cell tumor of bone (GCTB) is a benign neoplasm but occasionally shows local recurrence, and histologically consists of osteoclast-like giant cells (GC) and stromal mononuclear cells (SC), which are capable of proliferation and osteoblastic differentiation. Activation of Wnt signaling can induce osteoblast differentiation and osteoclastgenesis during bone resorption process. This study analyzed the profiles of β-catenin and cyclin D1 expression in GCTB to elucidate an involvement of Wnt pathway in tumorigenesis. We performed immunohistochemistry for β-catenin, cyclin D1, and Ki-67 in 16 GCTB tumors, including 5 recurrent cases that were surgically resected. All 16 cases of GCTB displayed β-catenin, cyclin D1, and Ki-67 expression. Immunoreactivity for β-catenin was observed in nuclei of SC and GC. Cyclin D1 immunoreactivity was found mainly in nuclei of GC, while Ki-67 immunoreactivity was restricted to nuclei of SC. The nuclear β-catenin labeling index (LI) in both SC (60.6 vs. 41.8%, $p = 0.074$) and GC (41.7 vs. 20.1%, $p = 0.095$) was higher in recurrent tumors than in primary tumors in all the 4 cases. However, Ki-67 LI in SC (18.8 vs. 19.9%, $p = 0.851$) and cyclin D1 LI in GC (55.4 vs. 70.1%, $p = 0.225$) were not higher in recurrent tumors than in primary tumors. Our results suggested activation of Wnt/ β-catenin pathway in GCTB tumorigenesis. Since cyclin D1 in GC was never associated with the expression of the well-known proliferative marker Ki-67, cyclin D1 expression might play a role in GC formation instead of promoting cell proliferation during GCTB tumorigenesis. Importantly, it was suggested that the nuclear β-catenin staining level might be associated with tumor recurrence in GCTB.

Keywords: Giant cell tumor of bone; β-catenin; Cyclin D1; Wnt pathway

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Introduction

Giant cell tumor of bone (GCTB), also referred to as osteoclastoma, is a benign but locally aggressive neoplasm of bone characterized by massive bone destruction at the epiphysis of long bone, which has a strong tendency to develop local recurrence. Histologically, GCTB consists of numerous scattered multinucleated osteoclast-like giant cells (GC), which are the characteristic hallmark of this tumor, and mononuclear stromal cells (SC), which represent the true neoplastic (proliferative) component [1]. Although their exact origin has not yet been defined, it is likely that SC might originate either from an osteoblastic lineage or from bone marrow mesenchymal cells, and might regulate the formation of GC in the neoplasm.

Cyclin D1 is a critical cell cycle regulator that drives the cell cycle from the G1 to the S phase. Increased nuclear cyclin D1 expression has been found in human tumors, including GCTB [2,3]. We have previously reported that cyclin D1 overexpression is significantly correlated with cytoplasmic β-catenin expression in thyroid tumors [4–6]. β-catenin is shown to be a key downstream effector of the Wnt signaling pathway to regulate cell growth/survival [7]. This pathway is activated by genetic mutations that stabilize the β-catenin protein, which accumulates in the cytoplasm, and then translocates to the nucleus. It then binds to the T-cell factor/lymphoid-enhancer factor (Tcf/Lef) [8–10] to activate genes, such as cyclin D1, and contributes to the oncogenesis of various human cancers [11–14].

Recent studies have implicated an important role for Wnt signaling in the regulation of skeletal function, and have suggested that activation of Wnt signaling may induce osteoblast differentiation and osteoclastgenesis during the bone resorption process [15–17]. This study analyzed the profiles of β-catenin and cyclin D1 expression in GCTB to elucidate an involvement of Wnt pathway in its tumorigenesis. In our results, we found an increased level of nuclear β-catenin in recurrent GCTB as compared to primary tumors.

Subjects and methods

Materials

A total of 16 cases of GCTB, including 11 primary and 5 recurrent tumors, were selected from a file of specimens surgically resected in our department between 1977 and 2006. These tissues were fixed in 10% buffered formalin and embedded in paraffin. Sections 4 μm in thickness were routinely stained with hematoxylin and eosin and prepared for immunohistochemistry. The clinical profile of each case, including Campanacci's radiographic grading system [18] and treatment, is summarized in Table 1.

Immunohistochemistry

After antigen retrieval, sections were immersed in 0.3% H_2O_2/methanol and subsequently preincubated with 10% normal goat serum. Then, tissues were incubated overnight at 4 °C with polyclonal anti-β-catenin antibody (GenWay Biotech, San Diego, CA) at a 1:50 dilution, monoclonal anti-cyclin D1 antibody (Zymed Labs, South San Francisco, CA) at a 1:50 dilution, or monoclonal anti-Ki-67 antibody (DAKO, Carpinteria, CA) at a 1:50 dilution. The slides were subsequently incubated with biotinylated goat anti-rabbit (for β-catenin) or anti-mouse IgG antibody (for cyclin D1 and Ki-67) for 1 h, followed by incubation with avidin-peroxidase for 30 min, and visualized with diaminobenzidine (DAB). A case of callus from a 17-year-old male patient was also used as a non-neoplastic tissue control. Control experiments included incubation with non-immunized rabbit serum (for β-catenin) or mouse serum (for cyclin D1 and Ki-67) instead of the primary antibodies. They did not show any staining.

Evaluation of immunohistochemical results

Tumor cells with nuclear and/or cytoplasmic staining were considered positive in immunohistochemistry for β-catenin, while only tumor cells with nuclear staining were considered positive in immunohistochemistry for cyclin D1 and Ki-67. The number of β-catenin, cyclin D1, and Ki-67 positive cells was counted in five different tumor areas at 200-fold magnification, and the percentage of immunoreactive cells from the total number of SC or GC was calculated as the labeling index (LI) in each case. For statistical analysis, the paired Student's *t*-test was used to assess differences in the β-catenin, cyclin D1, and Ki-67 LI between primary and recurrent tumors. Associations between the β-catenin, cyclin D1, and Ki-67 LI and Campanacci's radiographic grading were assessed using the Jonckheere–Terpstra test. A p-value of less than 0.05 was accepted as statistically significant.

Results

All 16 cases of GCTB displayed β-catenin, cyclin D1, and Ki-67 expression. Immunoreactivity for β-catenin was observed in nuclei of SC and in nuclei and/or cytoplasms of GC (Fig. 1A and D). Cyclin D1 immunoreactivity was found mainly in nuclei of GC and, occasionally, in a much smaller number of SC (Fig. 1B and E), while Ki-67 immunoreactivity was