Y-box binding protein-1 regulates cell proliferation and associates with clinical prognosis of osteosarcoma

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Introduction

Osteosarcoma (OS) is the most frequent malignant bone tumor occurring in young patients. Survival of the patients is improved remarkably by multi-agents chemotherapy and surgery. However, in spite of the extensive chemotherapy, the prognosis of advanced cases including distant metastasis and local recurrence is still dismal. Thus, development of new effective agents and/or other forms of therapy is desirable.

Y-box binding protein-1 (YB-1) is a multifunctional protein that can act as a regulator of transcription and translation. YB-1 is known as an important molecule that plays essential roles in drug resistance, DNA repair and other biological processes. Specifically, nuclear expression of YB-1 enhances expression of the ABC transporter gene, P-glycoprotein (P-gp), resulted in the increase of the P-gp-mediated drug resistance in various tumor cells. In addition, recent studies suggest the involvement of YB-1 in the regulation of the cell cycle and cell proliferation. In regard with the OS, we previously reported that the nuclear expression of YB-1 was closely associated with the expression of P-gp in OS cells (Oda, et al., 1998). However, the exact role of YB-1 in the clinical prognosis of OS remains unclear.

In this study, we investigated the effect of YB-1 knockdown on cell proliferation of OS cell lines. In addition, using by the immunohistochemical analysis, we further studied the association of the nuclear expression of YB-1 with clinical prognosis of OS patients.

Materials and Methods

Human OS cell lines, MG63, MNNG and SaOS, were used. Knockdown of YB-1 was carried out by using small interfering RNAs (siRNAs). The following double-stranded RNA 25-bp oligonucleotides were generated for YB-1 siRNA, 5'-UUUGCUUGGAUUUGCCUGGGACCC 3' (sense) and 5'-GGUCCUCCACGCAAUACCAGAAA 3' (antisense). Cells were transfected with siRNA using Lipofectamine2000 and Opti-MEM medium (Invitrogen) according to the manufacturer’s recommendations. The transfected cells were used in cell proliferation assays and flow cytometric analysis. Western blotting assays were performed to investigate whether YB-1 knockdown effected the expression of cell cycle regulating proteins, including cyclins and cyclin-dependent kinase inhibitors. To analyze the association of the nuclear expression of YB-1 with clinical prognosis of OS, immunohistochemistry was done with biopsy samples of 42 cases of OS.

Results

First, we examined the effect of YB-1 knockdown on cell proliferation of various OS cell lines. Treatment the cells with YB-1 siRNA decreased the expression of YB-1 protein in all cell lines tested. Proliferation of MG-63 and MNNG was strongly suppressed by YB-1 knockdown. In contrast, YB-1 knockdown did not affect the cell proliferation of SaOS2. Since YB-1 is known to regulate cell cycle, we then evaluated the effect of YB-1 knockdown on cell cycle by flow cytometric analysis. In OS cell lines, YB-1 knockdown induced a marked decrease of the population of cells in S phase and increased in the proportion of cells in the G1 fraction.

Next we investigated the effects of YB-1 knockdown on expression profiles of genes which regulate G1/S transition. Remarkably, in MG63 and MNNG, YB-1 knockdown decreased cyclin D1 and cyclin A which promote G1/S transition. These results suggested that YB-1 stimulated the progression of G1/S transition in OS cells.

Finally, we examined the association of nuclear expression of YB-1 with clinical prognosis of OS. The nuclear expressions of YB-1 was detected in 22 patients (52%) and that expression was significantly related to the poorer over all survival (Fig.1) (p<0.05, log-rank test).

Discussion

Recent studies showed that YB-1 knockdown by its cognate siRNA in culture inhibited the cell proliferation of various tumor cells, including breast cancer cells, prostate cancer cells and multiple myeloma cells. Moreover, YB-1 knockout mutation in mice caused a marked decrease in cell proliferation rates, resulted in embryonic lethality. These in vitro and in vivo results strongly suggest the direct involvement of YB-1 in the cell proliferation.

Consisting with this, we showed that YB-1 knockdown in OS cells inhibited the cell proliferation, along with the arrest of G1/S transition. Importantly, it has been shown that G1 phase deregulation is involved in formation and development of OS. Therefore, YB-1 might play critical roles in the oncogenesis of OS by promoting the G1/S transition of the cells. Interestingly, YB-1 knockdown did not affect the cell proliferation of SaOS2 that lacked p53 protein. Since YB-1 directly interacts with p53, and the interaction modulates the function of each protein, p53 would be indispensable for function of YB-1 in OS cells.

In conclusion, YB-1 regulated cell cycle progression at G1/S in human OS cell lines. Nuclear expression of YB-1 was also closely associated with the clinical prognosis of OS, indicating that YB-1 simultaneously could be a potent molecular target for treatment and prognostic biomarker for OS.

Figure 1. Overall survival of patients with osteosarcoma according to YB-1 nuclear expression.