Abstract. The correlation between mitogen-activated protein kinase (MAPK)/P53 signaling-dependent fatty acid synthase (FASN) expression and bone tumors was examined in the present study. We established the SH081 bone tumor cell line, which was used to determine the expression of FASN and MAPK/P53 at the mRNA and protein levels in bone tumor cells and normal cells. Compared with the normal cells, the expression of MAPK/P53 and FASN mRNA was significantly elevated, whereas inhibition of MAPK/P53 decreased FASN expression. Similarly, the expression of FASN and MAPK/P53 proteins were significantly elevated in the bone tumor cells and treatment with the MAPK/P53 inhibitor decreased the expression of FASN. To determine the role of MAPK/P53 in cell proliferation, bone cancer cells were treated with MAPK/P53 inhibitor and the results showed a reduced proliferation rate. Thus, FASN promotes the development of bone tumors and MAPK/P53 signaling contributes to bone tumors by upregulating the expression of FASN.

Introduction

Clinical statistics show that the bone is often involved in tumor metastasis during the late stages of most tumors (1). For example, the tumor cells of breast cancer, colon cancer, and lung cancer can be found in bone tissue (2). Clinical statistics also show that the osseous tumor metastasis would cause clinical symptoms (3) such as osteoporosis and pathological fracture by influencing sclerotin and joints (4). The incidence of osteosarcoma covers 45.3% of all types of bone tumors, and is the main morbidity among teenagers (5). Although research on bone tumors has made some progress, its nosogenesis remains to be elucidated, although its occurrence is influenced by genetic and environmental factors, such as nutrition, and exercise (6).

Materials and methods

Cell culture and treatment. The SH081 bone tumor cell line was established and maintained in our laboratory. We cultured the SH081 cell line at 3˚C, 5% CO₂, and collected the cells at 90% confluence. The cells were centrifuged for 10 min at 1,500 x g at 4˚C, and then the fluid was added and the cells were preserved at -80˚C. MAPK/P53 signaling pathway (100 µl, 100 ml) blocker (Calbiochem, Billerica, MA, USA) was added in observation group 2 during culture, and the cells were cultured at 37˚C, 5% CO₂. Other materials used were: dimethyl sulphoxide, fetal bovine serum and trypsin.

RT-qPCR. RNA extraction: We cultured the bone tumor cells at 37˚C, 5% CO₂, extracted total RNA and measured the amount of DNA (2). For qPCR the expression of MAPK/P53 and FASN mRNA in cells was analyzed. This study generated cDNA by reverse transcription of RNA as template for qPCR (Applied Biosystems Life Technologies, Foster City, CA, USA). The primer sequences are shown in Table I. Other materials used were: Transilluminator (Bio-Rad, Munich, Germany) and high speed and low temperature refrigerated centrifuge (Hitachi Ltd., Tokyo, Japan).

Enzyme-linked immunosorbent assay (ELISA) response. We followed the manufacturer's recommendation using the ELISA kit (Takara Bio, Dalian, China) with minor modifications (10).
Table I. qPCR primers.

| Primers | Primer sequences                          |
|---------|-------------------------------------------|
| MAPK/P53-F | GTCGATCGTCGATCGCTACGC                    |
| MAPK/P53-R | CGTAGCTAGTCGATCGACTAGC                    |
| FASN-F   | TGCTAGCTAGTCGATCGATCGTACG                |
| FASN-R   | CGTAGCTAGTCGATCGATCGTACG                |
| GAPDH-F  | TGCTAGCTAGTCGATCGAGTCAGTAC              |
| GAPDH-R  | CTGGGCTAGATCGACGAGGCTC                  |

MAPK, mitogen-activated protein kinase; FASN, fatty acid synthase; F, forward; R, reverse.

The standard protein sample was diluted in assay buffer at 1:50 and the standard curve was made according to the instruction. The samples were diluted in PBS (pH 7.2) at 1:100. We added 100 µl of the sample mix in each well, and added 50 µl of detection solution. TMB chromogenic substrate was used after the sample was incubated at room temperature for 2 h. Light absorption was measured at 495 nm, and the concentration of MAPK/P53 and FASN in each sample was calculated according to the standard curve.

Western blot analysis. The expression of APC in different samples was detected by western blot analysis. Total protein extracted with the animal cell total protein extraction kit was performed according to the ‘Guide of Molecular Clone’, 3rd edition. The primary antibody used was diluted at 1:800 and the secondary antibody was diluted at 1:500. Monoclonal and polyclonal, and animal origin are referred to a previous study (2).

Detection of cell proliferation by MTT. The procedure was performed as previously described (10,11).

Statistical analysis. Data were processed and analyzed by SPSS 20.0 (Chicago, IL, USA). When the test level was α=0.05, the difference was significant at P<0.05. When test level was α=0.01, the difference was significant at P<0.01.

Results

MAPK/P53 and FASN mRNA expression in normal and bone tumor cells. Using SH081 bone tumor cells and normal cells, total RNA was extracted to determine the expression levels of MAPK/P53 and FASN mRNA by qPCR. The expression level of MAPK/P53 mRNA in bone tumor cells was 8.74-fold higher than that in the normal cells, and the difference was statistically significant (Fig. 1). The expression levels of FASN mRNA in bone tumor cells was 13.3 times higher compared to normal cells, and statistically significant (Fig. 1). This result indicated robust upregulation of MAPK/P53 and FASN in bone tumors.

MAPK/P53 and FASN mRNA expression following MAPK/P53 inhibition. We then examined the consequence of inhibiting MAPK/P53 in bone tumor cells. MAPK/P53 and FASN mRNA levels were analyzed in treated and non-treated SH081 bone tumor cells by qPCR. The expression level of MAPK/P53 mRNA in the non-treated bone tumor cells was 7.05-fold higher than the level in bone tumor cells treated with MAPK/P53 inhibitor (Fig. 2). The expression levels of FASN mRNA in the non-treated cells was 11.4-fold higher compared to the SH081 cells treated with MAPK/P53 inhibitor (Fig. 2). These results indicated that MAPK/P53 regulates the expression of FASN gene in bone tumor cells.

Determination of MAPK/P53 and FASN expression using western blot analysis. To verify the changes in mRNA, we extracted total protein extracted from normal cells and SH081 bone tumor cells and measured the expression of the MAPK/P53 and FASN proteins by western blot analysis. The expression levels of MAPK/P53 and FASN proteins in normal cells were significantly lower than in the bone tumor cells (Figs. 3 and 4). This result was in accordance with the results observed by qPCR.

Expression of MAPK/P53 and FASN by ELISA. To verify the changes in MAPK/P53 and FASN proteins by a more
quantitative technique, we measured the expression by ELISA. The expression level of MAPK/P53 in SH081 bone tumor cells was 3.25±1.03 µl/l, but significantly higher in normal cells (1.21±0.76 µl/l) (Fig. 5). The expression level of FASN in normal cells was 2.03±0.96 µl/l, which was significantly lower than in bone tumor cells (7.52±1.25 µl/l) (Fig. 5).

Expression of MAPK/P53 and FASN proteins after MAPK/P53 inhibition by ELISA. The expression levels of MAPK/P53 and FASN in SH081 bone tumor cells treated and not treated with MAPK/P53 inhibitor were examined. The expression level of MAPK/P53 in bone tumor cells not treated with MAPK/P53 inhibitor was 3.39-fold higher than bone tumor cells treated with MAPK/P53 inhibitor (Fig. 6). The expression levels of FASN in bone tumor cells not treated with MAPK/P53 inhibitor was 6.03-fold higher than in bone tumor cells treated with MAPK/P53 inhibitor (Fig. 6). These results indicated that MAPK/P53 regulates the expression of FASN in bone tumor cells, which was consistent with the qPCR and western blot results.

FASN in SH081 bone tumor cells treated and not treated with MAPK/P53 inhibitor were examined. The expression level of MAPK/P53 in bone tumor cells not treated with MAPK/P53 inhibitor was 3.39-fold higher than bone tumor cells treated with MAPK/P53 inhibitor (Fig. 6). The expression levels of FASN in bone tumor cells not treated with MAPK/P53 inhibitor was 6.03-fold higher than in bone tumor cells treated with MAPK/P53 inhibitor (Fig. 6). These results indicated that MAPK/P53 regulates the expression of FASN in bone tumor cells, which was consistent with the qPCR and western blot results.

Proliferation of bone tumor cells treated with MAPK/P53 inhibitor. The cell proliferation of bone tumor cells treated and not treated with MAPK/P53 inhibitor was assessed to determine the functional relevance of the activation of the MAPK/P53 signaling pathway. Compared with the SH081 cells not treated with MAPK/P53 inhibitor, the cell proliferation of bone tumor cells treated with MAPK/P53 inhibitor was...
significantly reduced (Fig. 7). This result suggested that the MAPK/P53 signaling pathway promotes the proliferation of bone tumor cells.

Discussion

FASN in cells mainly participates in the metabolic process of acidic materials such as fatty acid (12). Moreover, FASN has various enzymatic activities such as condensation, dehydration, and polymerization in plants. Previously, it has been found that the pH of the bone tumor fluid was significantly lower than that in normal tissues. Analysis of the main constituents of bone tumor fluids shows that the content of acidic materials such as fatty acid is elevated, suggesting that in bone tumor the metabolism and synthesis of fatty acids and other acidic materials undergo pathological changes. The expression level of FASN in tumor cells such as breast and bladder cancer cells was apparently higher than that in normal cells (13). Breast cancer cells treated with FASN inhibitor exhibit a lower rate of proliferation and a higher ratio of apoptosis, which suggests that FASN regulates proliferation and apoptosis in breast cancer. Further research shows that in colon cancer cells FASN inhibits cell growth cycle transition from S to G2/M stage (10), promoting apoptosis (17). To the best of our knowledge, in breast cancer the MAPK signaling pathway impairs proliferation and apoptosis (14). Additionally, the expression level of MAPK in tumor cells was apparently higher than that in normal cells (15,16). Furthermore, inhibition of MAPK signaling in breast cancer impairs proliferation and promotes apoptosis (17). To the best of our knowledge, in the present study, we have shown for the first time that the expression level of FASN was significantly elevated in bone tumor cells compared to normal cells with the expression level of MAPK/P53. The expression level of FASN in tumor cells treated with MAPK/P53 inhibitor significantly decreased, and the proliferation rate was reduced. This indicates that FASN participates in bone tumor proliferation by regulating MAPK/P53 activity, and as a result participate in the occurrence and progress of bone tumors.

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