Sex Steroid Receptors Expression and Hormone-induced Cell Proliferation in Human Osteosarcoma

Introduction:

Gender differences in etiology have been reported in many disorders. Osteoporosis is one of them. As for osteosarcoma, however, there have been quite limited and insufficient reports about them. Sex steroid receptors including estrogen receptors (ER), progesterone receptors (PR), and androgen receptors (AR) have been sporadically reported in human osteosarcoma or its cell lines. Though, sex steroids have been considered to play some roles in human osteosarcoma but no systematic and detailed studies regarding the correlation between the status of these receptors in sarcoma cells and clinicopathological parameters have been reported.

From this point, we planned to examine the expression of Estrogen Receptor α, β (ERα, ERβ), Progesterone Receptor (PR), Androgen Receptor (AR) and aromatase in human osteosarcoma tissues, and to examine the effects of Estradiol, Progesterone, Androgen and receptor inhibitors of each sex steroid. Aromatase is an enzyme which converts serum androgens into estrogens in situ.

Methods:

The existence of ER, PR AR, and aromatase in 28 cases of osteosarcoma were examined using immunohistochemistry. These patients were 15 males and 13 females and their age ranged from 4 to 84 (average 24 ± 20 years old). Eighteen cases were associated with lung metastases and 14 cases died of lung metastases. All specimens were obtained from biopsies in Tohoku University Hospital from 1995 to 2006 before chemotherapies or irradiation, none of the cases were decalcified during the process of tissue preparation. All of these cases were retrieved from surgical pathology files of Tohoku University Hospital, Sendai, Japan. Research protocols for this study were approved by the Ethics Committee at Tohoku University Graduate School of Medicine (No. 2006-280).

Immunoreactivity of tumour cells was evaluated by employing an H-scoring system, as described by McCarty et al. with some modifications. Briefly, more than 500 tumor cells were counted in each case, and H-scores were subsequently generated by adding together 2 percentage weakly stained nuclei, giving a possible range of 0-200. The statistical significance was evaluated using t-test and P<0.05 was considered significant.

We then clarified the potential influence of sex steroids on cell proliferation of osteosarcoma cells using MG-63 human osteosarcoma cell line, which expressed all of these receptors, obtained from the Cell Resource Center for Biomedical Research, Tohoku University, Sendai, Japan. These cells were maintained in a RPMI-1640 (Sigma-Aldrich) with 10% FBS, and were preincubated for 48 hours with FBS-free medium prior to examination in order to remove exo-endogenous steroid hormones from the culture medium. Different concentrations of estradiol (E2; Sigma-Aldrich), progesterone (Sigma-Aldrich), or dihydrotestosterone (DHT; Wako Pure Chemical Industries) were added, and the assay was terminated after 3 days of culture by removing the medium from wells. Fulvestrant (ICI 182,780; Cat. No: 1047, Batch No: 10, Tocris Cookson Ltd.) as an ER inhibitor, mifepristone (RU 38,486; Sigma-Aldrich) as a PR inhibitor, or hydroxyflutamide (Tronto Research Chemical) as an AR inhibitor were added to the culture medium in order to specifically inhibit the actions of these sex steroids. The statistical significance was evaluated using Bonferroni/Dunn test and P<0.05 was considered significant.

Results:

Immunohistochemical study

Twenty three out of 28 cases of osteosarcomas demonstrated some or marked immunoreactivity of ERβ and 24 cases were associated with that of PR. Eight cases demonstrated immunoreactivity of AR. No cases demonstrated ERα and aromatase immunoreactivity. An average H-score for ERβ was 53 (range 0-125), PR 63 (range 0-167), and AR 6.0 (range 0-88). There were no correlation between the status of sex steroid receptors and age, sex, existence of pulmonary metastases in the patients examined, but there was a statistically significant correlation between H-score for ERβ and labeling index of Ki-67, suggestive of the correlation between estradiol and proliferation of osteosarcoma cells.

Cell culture study

1. Effects of E2 and fulvestrant on MG-63.

The cell proliferation of MG-63 was significantly stimulated by estradiol in the concentration of more than 1 nM (100.00 ± 6.52 in control, 109.67 ± 12.17 % in E2 of 1nM, 124.46 ± 8.70 % of 10nM, 127.28 ± 3.04 % of 100nM, and 136.52 ± 6.96 % of 1000nM), and was significantly suppressed by fulvestrant (100.00 ± 0.28 in control, 124.42 ± 0.58 in E2 of 10nM, 99.81 ± 8.93 in E2 of 10nM + ICI of 10nM, and 102.02 ± 3.75 in ICI of 10nM).

2. Effects of progesterone and mifepristone on MG-63.

The cell proliferation of MG-63 was significantly stimulated by progesterone in the concentration of more than 100 nM (100.00 ± 2.87 in control, 104.62 ± 2.87 % in progesterone of 1nM, 104.21 ± 3.28 % of 10nM, 110.87 ± 1.64 % of 100nM, and 122.77 ± 6.55 % of 1000nM), and was significantly suppressed by hydroxyflutamide (100.00 ± 2.87 in control, 122.77 ± 6.35 in progesterone of 10nM, 104.72 ± 2.66 in progesterone of 100nM + RU of 1nM, and 100.77 ± 3.89 in RU of 1nM).


The cell proliferation of MG-63 was significantly stimulated by DHT in the concentration of more than 100 nM (100.00 ± 6.52 in control, 108.27 ± 11.74 % in DHT of 1nM, 109.91 ± 10.87 % of 10nM, 116.30 ± 8.70 % of 100nM, and 117.88 ± 5.21 % of 1000nM), and was significantly suppressed by hydroxyflutamide (100.00 ± 2.87 in control, 116.73 ± 7.78 in progesterone of 100nM, 99.42 ± 6.63 in progesterone of 100nM + flutamide of 10nM, and 100.63 ± 5.19 in flutamide of 10nM).

Discussion:

As for sex steroid receptors in human osteosarcoma tissue, Walker et al. examined 4 Osteosarcomas. But there has been no report since then. In our study, ERβ and PR were positive in most cases, and AR in some cases. Therefore, osteosarcoma may be sex steroid hormone-dependent tumor. In our immunohistochemical study, there was significant positive correlation between H-score of ERβ and labeling index of Ki-67. This fact implies estrogen may control proliferation of human osteosarcoma cells. In MG-63, Zhuang et al., MacNamara et al., and Luo et al. clarified the existence of AR, PR, ERα and β, respectively. In our cell culture study, by addition of estradiol, progesterone, and dihydrotestosterone, respectively, cell proliferation of MG-63 was significantly stimulated. Furthermore, by addition of these inhibitors simultaneously, cell proliferation was significantly inhibited. These facts indicated the possibility of hormone therapy for human osteosarcoma.

As there was no aromatase in human osteosarcoma tissues, there was no estrogen synthesis in tumor tissues, and estrogen in circulatory blood may be important. There have been no reported studies of employing the endocrine therapy to the patients of osteosarcoma to the best of our knowledge, but these studies should be considered worthwhile to pursue considering the relatively poor clinical outcome of osteosarcoma patients with pulmonary metastases.

References:

1) McCarty Jr KS, Miller LS, Cox EB, Konrath J, McCarty Sr KS.


