INHIBITION OF TUMOR GROWTH AND LUNG METASTASIS OF OSTEOSARCOMA IN VIVO BY HYALURONAN OLIGOSACCHARIDES

Kozo Hosono, Yoshihiro Nishida, Warren Knudson, Naoki Ishiguro

Department of Orthopaedic Surgery, Graduate School of Medicine, Nagoya University
65 Tsurumai-cho, Showa, Nagoya 466-8550, Japan

Department of Biochemistry, Rush Medical College, 1653 W. Congress Parkway, Chicago, IL 60612, USA

Introduction
Hyaluronan (HA) is a large, linear, negatively charged polysaccharide with molecular weights ranging 10^5-10^7, interacting with pericellular matrix, and regulating tumor cell biology, such as proliferation, motility, and invasiveness. Manipulation of HA synthesis alters the hyaluronan-rich-matrix and malignant properties (1). Numerous studies suggest that HA interacts with specific cell surface receptors, such as CD44 and RHAMM, modulating cell behavior. Perturbation of endogenous hyaluronan-tumor cell interaction by administration of HA oligosaccharides results in suppression of tumor growth (2, 3).

Osteosarcoma is one of the most common primary malignant bone tumors. Chemotherapy has improved the prognosis of osteosarcoma patients remarkably; however, still not a few patients develop distant metastasis. New therapeutic tools should be introduced for the treatment of osteosarcoma. Although exogenous HA oligosaccharides is reported to inhibit the proliferation and invasiveness of osteosarcoma cells in vitro (4), effects of HA oligosaccharides on in vivo tumor growth and distant metastasis has not been investigated. The aim of this study is to determine the effects of various size of HA oligosaccharides on tumor growth and lung metastasis of osteosarcoma in vivo, and furthermore the distribution of exogenous HA, interaction with CD44, effects on apoptotic activity are also analyzed.

Material and Methods
Preparation of HA oligosaccharides: HA was depolymerized by partial digestion with testicular hyaluronidase and separated into size uniform HA oligosaccharides by anion exchange chromatography after removal of hyaluronidase. The purity and size of each HA oligosaccharide was confirmed by using HPLC analyses. Endotoxins, proteins and DNA of hyaluronidase. The purity and size of each HA oligosaccharide was analyzed.

HA oligosaccharides by anion exchange chromatography after removal of HA oligosaccharides on in vivo tumor growth and distant metastasis has not been investigated. The aim of this study is to determine the effects of various size of HA oligosaccharides on tumor growth and lung metastasis of osteosarcoma in vivo, and furthermore the distribution of exogenous HA, interaction with CD44, effects on apoptotic activity are also analyzed.

Effects of HA oligosaccharides on tumor growth and lung metastasis in vivo: Human osteoblastic osteosarcoma cell line, MG-63, cannot be grown in nude mice. Murine high metastatic osteosarcoma cell line, LM8, was inoculated subcutaneously into the back of C3H/He mice at concentration of 2x10^6 cells/200 µl, and allowed to grow for a period of 14 days when a tumor mass was palpable at the back of mice. HA4 (250 µg/ml, 100 µl), HA8 (250 µg/ml, 100 µl), HMWHA (250 µg/ml, 100 µl), and PBS (100 µl) as control were injected into the tumor mass once a day from day 14 to day 24. At day 28, mice were sacrificed, and local tumor was wet-weighed, formalin-fixed and paraffin-embedded. TUNEL assay was performed according to the manufacturer’s instruction. Significance among different experimental groups. P value less than 0.05 was considered as significant.

Discussion
In this study, HA8 had inhibitory effects on both local tumor growth and lung metastasis, whereas HA4 and HMWHA did not. We demonstrated that HA oligosaccharides with length more than 8mers has inhibitory effects on pericellular matrix formation, cell proliferation, and invasiveness in vitro (4), however, the effects in vivo has not been well known, especially on osteosarcoma cells. The results of this study in vivo were in accordance with previous in vitro data. The limitation of this study is the lack of analysis of these suppressive effects, such as inhibition of neovascularization, down-regulation of matrix metalloproteinase activity. And other osteosarcoma cell line, which has less pericellular matrix, should be investigated with treatment of HA oligosaccharides. Manipulation of pericellular collagen-rich matrix in malignant cells has been shown to be a candidate for therapeutic tools, such as antitumor agents (5). Treatment of exogenous HA oligosaccharides might be one of the tool to control osteosarcoma cells. However, other groups reported the controversial results that HA oligomers stimulate tumorogenicity (6). The discrepancy of the results might be due to the difference of the cells, methods for preparation of HA oligosaccharides. Further investigation, such as different cell line of osteosarcoma, should be necessitated for practical use of this reagent.

Statistical analysis: Student’s t test was used to examine the statistical significance among different experimental groups. P value less than 0.05 was considered as significant.

Results
Effects of HA oligosaccharides on tumor growth and lung metastasis: HA8 has been shown to have inhibitory effects on cell proliferation and invasiveness in vitro partly via suppression of pericellular matrix formation, whereas HA4 does not (4). As same way as in vitro, exogenous HA8 inhibited local tumor growth significantly (P<0.05) compared to control, whereas HA4 did not show the inhibitory effect.

Detection of exogenous HA oligosaccharides: Pericellular matrix formation and invasion in MG-63 was suppressed with treatment of exogenous HA34, and this HA oligosaccharides distributed on cell surface after treatment for 24hrs. Preincubation of MG-63 with Hermes-1, anti-CD44 monoclonal antibody, perturbed the distribution of exogenous HA34 on cell surface dose of Hermes-1 dependently.

Effects of HA oligosaccharides on apoptotic activity: TUNEL assay demonstrated that the number of apoptotic cells was significantly higher with treatment of HA8 than other treatment (P<0.01).

References
1: Itano N et al, Cancer Res 59; 2499-2504, 1999
2: Sugahara KN et al, J Biol Chem 278; 32259-32265, 2003
4: Hosono K et al, Trans Orthop Res Soc 30;435, 2005

Statistical analysis: Student’s t test was used to examine the statistical significance among different experimental groups. P value less than 0.05 was considered as significant.

Results
Effects of HA oligosaccharides on tumor growth and lung metastasis: HA8 has been shown to have inhibitory effects on cell proliferation and invasiveness in vitro partly via suppression of pericellular matrix formation, whereas HA4 does not (4). As same way as in vitro, exogenous HA8 inhibited local tumor growth significantly (P<0.05) compared to control, whereas HA4 did not show the inhibitory effect.

Detection of exogenous HA oligosaccharides: Pericellular matrix formation and invasion in MG-63 was suppressed with treatment of exogenous HA34, and this HA oligosaccharides distributed on cell surface after treatment for 24hrs. Preincubation of MG-63 with Hermes-1, anti-CD44 monoclonal antibody, perturbed the distribution of exogenous HA34 on cell surface dose of Hermes-1 dependently.

Effects of HA oligosaccharides on apoptotic activity: TUNEL assay demonstrated that the number of apoptotic cells was significantly higher with treatment of HA8 than other treatment (P<0.01).

Discussion
In this study, HA8 had inhibitory effects on both local tumor growth and lung metastasis, whereas HA4 and HMWHA did not. We demonstrated that HA oligosaccharides with length more than 8mers has inhibitory effects on pericellular matrix formation, cell proliferation, and invasiveness in vitro (4), however, the effects in vivo has not been well known, especially on osteosarcoma cells. The results of this study in vivo were in accordance with previous in vitro data. The limitation of this study is the lack of analysis of these suppressive effects, such as inhibition of neovascularization, down-regulation of matrix metalloproteinase activity. And other osteosarcoma cell line, which has less pericellular matrix, should be investigated with treatment of HA oligosaccharides. Manipulation of pericellular HA-rich matrix in malignant cells has been shown to be a candidate for therapeutic tools, such as antitumor agents (5). Treatment of exogenous HA oligosaccharides might be one of the tool to control osteosarcoma cells. However, other groups reported the controversial results that HA oligomers stimulate tumorogenicity (6). The discrepancy of the results might be due to the difference of the cells, methods for preparation of HA oligosaccharides. Further investigation, such as different cell line of osteosarcoma, should be necessitated for practical use of this reagent.

Fig1. Effects of HA oligosaccharides in vivo.
A: tumor weight, *P<0.05, B: number of lung metastasis, *P<0.05

52nd Annual Meeting of the Orthopaedic Research Society
Paper No: 1845