PERICELLULAR MATRIX FORMATION ALTERS THE EFFICIENCY OF CELLULAR UPTAKE OF NFkB DECOY IN OSTEOSARCOMA CELLS

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Introduction
Nuclear factor-kB (NFkB) is a transcriptional factor which plays significant roles in tumorigenicity. NFkB decoy is a synthetic double-strand oligo-DNA containing a sequence corresponding to the consensus of the NFkB binding sites (1). Inhibition of NFkB activation by using NFkB decoy could induce apoptosis and reduce chemoresistance in cancer cell line (2), and also inhibit osteosarcoma cell invasion and motility (3). However, the lack of specificity to the cell and low efficiency of cellular uptake are two major problems. Osteosarcoma is the most common primary malignant tumor of bone. Despite aggressive chemotherapy and radical resection of the tumor, significant proportion of patients eventually develop pulmonary metastasis and succumb to their disease. Therefore, there is a pressing need to develop new and alternative approaches to the current medical treatment of osteosarcoma. More understanding of the basic biology, such as the roles of tumorous extracellular matrix (ECM), may provide novel tools for treatment. Recent studies have suggested that ECM could provide protection against anti-tumor drugs and host immunocompetent cells (3, 4). The aim of this study is to analyze the relationship between the formation of tumorous ECM and the efficiencies of cellular uptake of NFkB decoy in osteosarcoma cells.

Material and Methods
Chemicals: NFkB decoy (Anges MG Inc. Osaka, Japan) used in this study are phosphorothioate double stranded oligonucleotides. Cell culture: The human osteosarcoma cell lines, HOS and MG-63 (the American Type Culture Collection, VA), were cultured in DMEM containing 10% fetal bovine serum (FBS) and antibiotics. Particle exclusion assay: Cells were plated and cultured for 24 h or 48 h. After 24 h or 48 h, the medium was removed and replaced with suspension of FITC-labeled decoy oligonucleotides in PBS containing 0.1% BSA. The cells were allowed to settle for 15 min. The cells were observed and photographed, and the area of pericellular matrix was measured with image analysis software (NIH Scion image). Fluorescent microscopy: HOS and MG-63 cells were seeded on chamber slide and were incubated for 24 h or 48 h. After 24 h or 48 h, the culture, the supernatant medium was replaced with 1.0 μM FITC-labeled decoy oligonucleotides in DMEM, and further incubated for 12 h. The cells were washed with PBS and fixed with 2% paraformaldehyde, and scanned using a fluorescent microscope (BX60, Olympus, Inc., Tokyo, Japan). Flow cytometric analysis: After 24 h or 48 h, the culture, the supernatant medium was replaced with 1.0 μM FITC-labeled decoy oligonucleotides in DMEM. After 12h, cells were harvested with trypsin-EDTA 0.25%, and then washed with PBS. After the wash with PBS, the cells were resuspended in 2% paraformaldehyde and analyzed by flow cytometry. Enzymatic removal of ECM: MG-63 cells were seeded on chamber slides and were incubated for 48 h. After 48 h culture, the cells were washed with PBS and the cells were treated with 2 units/ml Streptomyces hyaluronidase (St-HA’ase) (Sigma) for 1 h at 37°C. After the treatment with St-HA’ase, the cells were washed with PBS and the medium was replaced with 1.0μ M FITC-labeled decoy oligonucleotides in DMEM. The cells were cultured for a further 12h and subjected to the analysis for decoy uptake by fluorescent microscopy.

Results
Formation of tumorous ECM: As can be seen in Fig. 1, MG-63 cells exhibited abundant pericellular ECM compared with HOS cells. Uptake of FITC-labeled NFkB decoy: At 24h time point, fluorescent was observed both in the cytoplasm and nucleus. There was no difference of uptake between HOS and MG-63 cells. On the other hand, at 48h, difference could be seen between them. In MG-63 cells, fluorescence was not observed in the nucleus and the intensity decreased compared to the cells at 24h. Whereas, in HOS cells, there was no difference in the distribution and fluorescent intensity between 24h and 48h culture (Fig.2). The fluorescent intensity determined with flowcytometry decreased in MG-63 cells at 48h compared with 24h, whereas there was no difference in HOS cells between 24h and 48h culture (Fig.3). Effects of St-HA’ase on cellular uptake of FITC-labeled NFkB decoy:

Following the St-HA’ase treatment, the amount of fluorescent uptake increased remarkably compared with the cells without enzyme treatment.

Discussion
It is well known that the efficiencies of cellular uptake of naked NFkB decoy is low, because of the problems such as lack of specificity to the target cells and its’ stability. To the best of our knowledge, no research has yet been carried out analyzing the efficiencies of cellular uptake of NFkB decoy concerning ECM formation. In this study, it was demonstrated that the efficiencies of cellular uptake of NFkB decoy decreased in MG-63 cells with abundant ECM formation with time but not in HOS cells with poor ECM formation. Furthermore, after enzymatic removal of ECM in MG-63 cells, intracellular concentration and localization of FITC-labeled NFkB decoy in nucleus increased. These results suggest that rich pericellular matrix might disturb the cellular uptake of NFkB decoy. Although further experiment will be necessary, these results would provide the basic information about the cellular uptake mechanism of NFkB decoy, especially for ECM-rich cells.

References