MDA231 breast cancer cells modulate the MMPs expression in osteoblasts

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INTRODUCTION:
Breast cancer often metastasizes to bone and most patients with breast cancer have bone metastases, which generally are osteolytic lesions. Since matrix metalloproteinases (MMPs) are enzymes that degrade structural components of the extracellular matrix to create space for cells to migrate or produce specific substrate-cleaveagge fragments, which is important in cancer progression. Osteoblasts and osteoclasts can produce MMPs, which are involved in bone degradation, and it has been reported that MMPs were necessary for PTH to enhance bone resorption. We here examined the MMPs expression in osteoblasts treated with cancer conditioned medium. The treatment of cancer CM in osteoblasts increased the expression of MMP9 and MMP13 time dependently and dose dependently. The effect were counteracted by the addition of PD98059 (ERK inhibitor), SB203580 (MEK inhibitor), and wortmannin (PI3K inhibitor). In addition, it is found that PTHrP may be involved in the regulation of MMPs in osteoblasts by cancer conditioned medium.

METHODS:
MDA231 were human breast cancer cell line with a high potency of metastasis. Cells were cultured in RPMI6400 supplemented with 10% FCS and 100 IU/ml penicillin at 37°C in a humidified atmosphere with 5% CO2 in air.

Primary osteoblasts were obtained from the calvaria of fetal rats. The calvaria were cut into small pieces and were treated with 1mg/ml collagenase solution for 20-30 minutes at 37°C. The next two 20 minute sequential collagenase digestions were then pooled and filtered through 70µm nylon filters. The cells were grown on the plastic cell culture dishes in 5% CO2.

MDA231 cells were cultured in different density (1:10:100:10^3:10^4:10^5) for 24hr. The conditioned media were prepared by culturing the cells in 1% FBS medium, and the supernatant was collected as cancer condition medium (CM) 24hr later.

Treatment of cancer CM on osteoblast for 6hr, mRNA of osteoblasts was analyzed by Reverse Transcription Polymerase Chain Reaction (RT-PCR). Total RNA was extracted from primary osteoblasts by using TRizol kit after 1-3 days of treatment. RNA was analyzed by using one-step SuperScriptIII and Taq polymerase. Amplification was accomplished with 28-37 cycles. PCR products were then separated electrophoretically in a 2% agarose DNA gel and stained with ethidium bromide.

Treatment of osteoblast with cancer CM for 24hr, proteins were prepared using a sequential extraction protocol. MMP13 immunoblots were performed on lysate from cultured osteoblasts. Protein concentration was determined using the bicinchoninic acid protein assay. Thirty micrograms of protein extract were loaded in each lane of a 8% SDS-Polyacrylamide gel and run at 120 V. Protein was transferred to a nitrocellulose membrane at 22V (80mA) overnight at 4°C. After blocking, the membrane was incubated with an anti-rat MMP13 antibody overnight at 4°C and then was washed by PBST. After washing, the membrane was incubated with a HRP-conjugated secondary antibody for 1h at room temperature, followed by three 15-min washings. The labeling was visualized by addition of chemiluminescence reagent.

MDA231 cells were cultured on 6-well plate to <50% confluence and transfected with siRNA pool using Oligofectamine reagent (Invitrogen) in serum and antibiotic-free media for 6 h at 37°C. Cells were allowed to recover with serum without removing the transfection mixture and incubated for an additional 24 h. The following 24hr, cells were changed with 1% FBS medium, and the supernatant were collected to treat on the osteoblasts. The values given are means ± S.E.M. The significance of difference between the experimental group and control was assessed by Student's t test. The difference is significant if the p value is less than 0.05.

RESULTS:

Treatment of osteoblasts with MDA231 conditioned medium upregulate the expression of MMP9 and MMP13 time-dependently (Fig 1A) and dose-dependently (Fig 1B). The effect were counteracted by the addition of PD98059, SB203580, and wortmannin (Fig 1C). When the conditioned medium were collected from the MDA231 which silencing the PTHrP, the upregulation of MMP in osteoblasts was reversed. (Fig 2)

DISCUSSION:
Breast cancer predominantly causes osteolytic bone metastasis. Once cancer cell enter the bone, it secret factors that act on bone and other cells within the skeleton, which develop the vicious cycle. In our study, we found that cancer cells upregulate the expression of metalloprotease in osteoblasts through the PTHrP pathway. The MMP-13 may be involved in the bone resorption of cancer cells.

REFERENCES:
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ACKNOLEDGEMENT: