Enhancement of ABC transporter expression by osteopontin in prostate cancer

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INTRODUCTION:
Osteopontin (OPN), a noncollagenous bone extracellular matrix protein, is a secreted adhesive glycoprotein with a functional RGD cell-binding domain that interacts with the αvβ3 cell surface integrin heterodimer. OPN has been reported to be overexpressed in human cancers, and OPN overexpression confers malignant transformation and has been observed frequently in a variety of tumorigenic human cell lines. Human prostate cancer has the propensity to metastasize to the bone where reciprocal cellular interactions between prostate cancer and bone cells are known to occur. In the cancer chemotherapy, drug resistance is a major obstacle, which is associated with overexpression of ATP-binding cassette (ABC) drug transporters that actively pump out a variety of amphipathic compounds to cause multidrug resistance (MDR). In this study we investigate the role of osteopontin during chemotherapy in regulating ABC transporter-MDR1 expression.

MATERIALS AND METHODS:
PC3 is a human androgen-independent prostate cancer cell line with a high potency of bone metastasis. Cells were cultured in RPMI1640 supplemented with 10% FCS and 100 IU/ml penicillin at 37°C in a humidified atmosphere with 5% CO₂ in air.

After treatment of osteopontin, mRNA of PC3 was analyzed by quantitative real-time PCR. Total RNA was extracted by using TRizol kit (MDBio, Inc., Taipei, Taiwan). RNA was analyzed by using two-step SuperScriptIII (Promega; Madison, WI) and Taq polymerase. Real-time PCR was performed using Tagman Master Mix and analyzed with a StepOne Plus™ (Applied Biosystems).

After treatment of PC3 with osteopontin, protein was prepared using a sequential extraction protocol. P-glycoprotein immunoblots were performed on lysate from PC3. Protein concentration was determined using the bicinchoninic acid protein assay. Equal proteins were separated using the SDS-polyacrylamide gel and transferred to polyvinylidene difluoride membranes. After blocking, the membrane was incubated with indicated antibody overnight. After PBST washing, the membrane was incubated with a HRP–conjugated secondary antibody for 1h. The blots were visualized by enhanced chemiluminescence reagent.

Cell viability was assessed by MTT assay. Culture medium was aspirated 24 hr after treatment and MTT (0.5 mg/ml) was added to each well. MTT was then removed 30 min later and cells were lysed by 200μl DMSO. OD values at 550 nm and 630 nm were measured by using microplate reader (Bio-Tek, Winooski, VT).

PC3 cells cultured on 24-well plates were treated with osteopontin for 60min before treatment with daunomycin. After 24 hr, the daunomycin, a natural fluorescent anthracycline antibiotic, uptake was observed using confocal microscopy.

The data are given as mean ± 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 PC3 with osteopontin upregulates the expression of MDR1 mRNA (Fig 1A and 1B) and p-glycoprotein protein level (Fig 1C and 1D) time-dependently and dose-dependently. Daunomycin, a natural fluorescent anthracycline antibiotic, was transported into cytoplasm, and observed by confocal microscope. Treatment of osteopontin inhibited the uptake of daunomycin. It indicates that daunomycin is pumped out by p-glycoprotein, which is induced by osteopontin. The inhibitory effect of osteopontin is antagonized by anti-αvβ3 mAb (Fig 2). In cell viability assay, daunomycin induced PC3 cell death dose-dependently (Fig 3A), which was markedly antagonized by osteopontin (10 ng/ml). Pretreatment of anti-αvβ3 antibody for 60 min effectively inhibited the action of osteopontin (Fig 3B).

DISCUSSION:
Osteopontin is a functional protein which mediates cell adhesion, chemotaxis, stress-dependent angiogenesis, and tumor cell metastasis, and OPN was expressed in a variety of tissues, including bone, vascular, and tumor. It fuctions by mediating cell-matrix interactions and cellular signaling through binding with integrin and CD44 receptors. In this study we found that OPN, a noncollagenous bone matrix protein, up-regulates MDR1 expression to induce drug resistance after treatment with daunomycin in PC3 cells, a chemotherapy substrate of MDR1, through αvβ3 integrin. OPN may be an important role in and drug resistance during chemotherapy.

REFERENCES: