Versican Modulates Pericellular Matrix Formation and Tumorigenecity in Swarm Rat Chondrosarcoma Cells

INTRODUCTION:
Versican is a large chondroitin sulfate proteoglycan (CSPG). It is one of extracellular proteoglycans that bind to hyaluronan (HA) of which aggrecan, the cartilage-specific proteoglycan is the prototype. Previous studies reported that versican expression is correlated with tumor progression in several types of cancer. (1) Our preliminary study indicated that versican did not express in low grade, but expressed in high grade chondrosarcoma tissues, and swarm rat chondrosarcoma cells did not synthesize versican but aggrecan. We hypothesize that versican expression in swarm rat chondrosarcoma cells by genetic modification may alter matrix formation and tumorigenecity of the cells. Alternative splicing of versican generates at least four isoforms named V0, V1, V2 and V3. In this study, we generated stable transfectants of versican V1 and V3 isoform in swarm rat chondrosarcoma cells and report that V1 isoform modulates pericellular matrix formation and alters cell proliferation, motility and invasiveness.

METHODS:
Cell cultures: Human versican splicing variant 1 (V1-RCS), variant 3 (V3-RCS), or only GFP (GFP-RCS) were stably transfected to swarm rat chondrosarcoma cells by Trap-In System, a new gene expression system combining promoter trapping and site-specific gene integration methods.

Proliferation assay: Each cell line was seeded in 96-well plates at 5x10^3 cells/well. At 24, 48 and 72h cell growth was measured by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (Boeringer Mannheim, Germany).

Motility and matrigel invasion assays: Chemotactic motility of each cell lines was investigated using 24-well cell culture chambers containing inserts with 12µm pores. Invasion of each cell lines was assayed in the same chambers that also contained a Matrigel layered on a 12µm pore membrane. Migrating and invading cells on the lower surface of the membrane were counted under the light microscopy.

Visualization of pericellular coat: Pericellular coat formation was visualized by a particle exclusion assay. Pericellular coat formations were quantified from digital photographs using Scion image software. The area delimited by red blood cells and the area delimited by the cell membrane was measured to give a coat to cell ratio.

Immunocytochemistry: Immunocytochemistry of each cell lines were performed on chamber slides by immunostaining using avidin-biotin immunoperoxidase technique. Three primary antibodies were used: 1) a monoclonal antibody against versican (2B1, Seikagaku, Japan); 2) a monoclonal antibody against aggrecan (BC-14, Abcam, U.S.A.); 3) biotinylated Hyaluronic Acid Binding Protein (HABP, Seikagaku, Japan).

Real time RT-PCR: Expression of aggrecan mRNA level of each cell lines was assessed by real time RT-PCR method. Gene expression was quantified by dividing the level of aggrecan mRNA expression by the level of GAPDH mRNA expression. Statistical analysis: ANOVA was used to assess differences between means. P-values of <0.05 were considered statistically significant. All analyses were performed using SPSS 11.0 for Windows software.

RESULTS:
Cell proliferation: The differences in cell viability between each cell lines were not statistically significant at 24h and 48h after incubation, but at 72h after incubation, only V1-RCS was significantly higher viability than RCS (P < 0.001). Migration and Invasion assay: V1-RCS cells exhibited a statistically significant higher motility and invasiveness than RCS cells. On the other hand, there was no significant difference between V3-RCS and RCS (Fig. 1). Formation of pericellular coat: V1-RCS cells produced more extensive pericellular coat than other cell lines (Fig. 2). The average of 50 cells in V1-RCS was statistically significant higher than that of RCS cells (P=0.011).

Expression of versican, aggrecan and Hyaluronan: As 2B1 recognized only human versican, RCS and GFP-RCS cells did not show positive staining for 2B1. On the other hand, positive immunostaining for versican was observed mainly in cell membrane, cytoplasm and extracellular area of V1-RCS cells and in the cytoplasm of V3-RCS cells. Aggrecan was weakly immunolocalized to the cell membrane and cytoplasm in all cell lines. HABP was observed mainly in cell membrane of all cell lines and pericellular area of V1-RCS cells (Fig. 3).

Expression levels of aggrecan mRNA: Aggrecan mRNA expression was detected in all cell lines. Interestingly, expression levels of aggrecan mRNA in V1-RCS and V3-RCS cells was significantly lower than in RCS cell.

DISCUSSION:
This study showed overexpression of versican V1 isoform stimulates a pericellular coat formation, which may result in the up-regulation of cell proliferation, motility and invasion. Considering that both aggrecan and versican are hyaluronan binding proteoglycans, increased pericellular coat by versican V1 isoform will be more tumorigenic. Overexpression of V1 isoform stimulates tumorigenecity, whereas V3 isoform does not. A possible explanation may be the difference of attached glycosaminoglycan between these two variants expressing cells. (2) A limitation of this study was to use rat versican cDNA but human versican cDNA to transfect. However, there was highly homologous between human and rat versican cDNA and human versican may have a similar function for RCS cells. Although we should further analyze the function of versican also in vivo, the results of this study indicates versican might be a possible therapeutic target in high grade chondrosarcoma.

REFERENCES:
2: Lemire J et al, J Cell Physiol 190; 38-45, 2002