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Functional Analysis of Cell Surface Heparan Sulfate Proteoglycans as Modulators of MMPs

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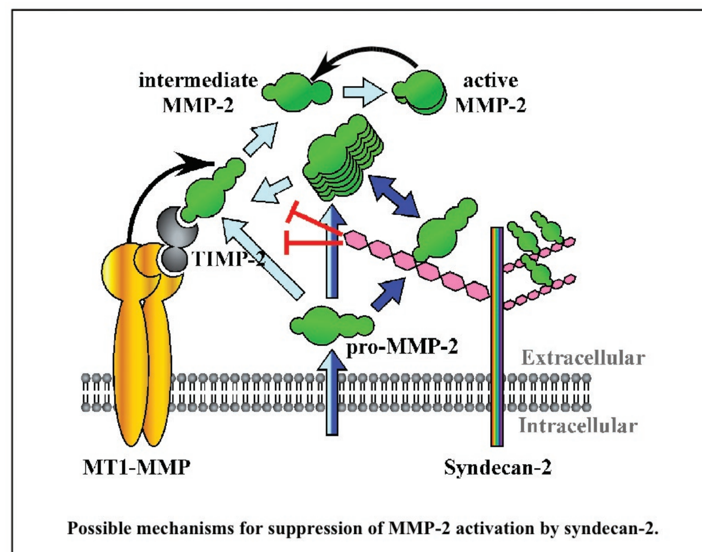
I graduated from Tokyo Metropolitan University, Tokyo, in 1976 and received PhD in developmental biology. 1975-1978, Pre- and Postdoctoral fellow at Mitsubishi-Kasei Institute of Life Sciences, Tokyo. 1978-2003, Researcher at Clinical Research Institute, National Nagoya Hospital, Nagoya. 2003-present, Chief at Clinical Research Center, National Hospital Organization Nagoya Medical Center, Nagoya.

My scientific interest is "functions of proteoglycan in cell-ECM interaction" consistently since the school days. Recently I have analyzed functional specificity of syndecans. Seemingly fuzzy characters of proteoglycan fascinate me. It is just the femme fatale who brings me delight, trouble or unhappiness. Anyway, my study has evolved brilliantly year after year. It is evident from the transition history of materials that I have dealt with. I started my career as a researcher of proteoglycan in developing sea urchin embryos. And then chick embryos, mouse, and finally human cells, EVOLUTION.



Possible mechanisms for suppression of MMP-2 activation by syndecan-2.

It has been demonstrated that cell surface heparan sulfate proteoglycans act as receptors for both soluble and insoluble heparin-binding molecules which contribute to compose the extracellular microenvironment, and function in various signal transductions of "outside-in" or "inside-out". Here we report a new function of cell surface heparan sulfate proteoglycans that act as a receptor for matrix metalloproteinases (MMPs). Using several mouse tumor cell clones, we have analyzed the participation of cell surface heparan sulfate proteoglycans in tumor metastasis and invasion in relation with MMPs.

By Lewis lung carcinoma-derived tumor clones with different metastatic potentials, we found an inverse correlation between an expression level of syndecan-2, a transmembrane-type heparan sulfate proteoglycan, and a metastatic potential of the

clones, and confirmed that this correlation was causal relationship by a manipulation of expression levels of syndecan-2. The continuous study to elucidate the mechanism underlying this correlation revealed that syndecan-2 functioned as a suppressor of MMP-2-activation. MMP-2 (gelatinase A) is a degradation enzyme of extracellular proteins, especially type IV collagen, and is known to be correlated directly with tumor metastasis. The activation of MMP-2 on cell surface has been well documented, that is, MMP-2 is secreted in a latent form, bound to cell surface through MT1-MMP and TIMP-2 complex, and then cleaved to be released as an active form by another MT1-MMP (Fig). Interestingly, no significant difference in expression levels of MMP-2, MT1-MMP and TIMP-2 among the tumor clones was observed, but the activation degree of MMP-2 was nevertheless in proportion to metastatic potential and in inverse proportion to expression level of syndecan-2. This was confirmed by the transfection of cDNA of syndecan-2 core protein to the highly metastatic clone with low syndecan-2 expression. It was of note that the metastatic potential of the transfectant was dramatically decreased. It became clear by the following experiments that this regulation was controlled through heparan sulfate side chains of syndecan-2. 1) The enzymatic removal of heparan sulfate from cell surface of the low metastatic clone promoted the activation of MMP-2. 2) The transfection of mutant cDNA of syndecan-2 core protein lacking glycosaminoglycan-elongation sites into the high metastatic clone did not affect both on MMP-2 activation and on metastatic potential. 3) Furthermore, it was demonstrated that MMP-2 exhibited a strong binding activity with high avidity to heparin. The results obtained indicate that syndecan-2 acts as suppressor receptor of MMP-2 activation on cell surface as shown in the figure.

In another system of Colon 29-derived adenocarcinoma clones with different metastatic potentials, we found that the expression and activity of MMP-9 (gelatinase B) of the highly metastatic clone were significantly higher than those of the low metastatic clone, although those of MMP-2 were not different between the clones. The former clone showed higher ability on invasion to adjacent muscle tissues as compared to the latter. *In vitro* behaviors of the clones, such as degrees of migration on fibronectin-substratum or invasion through Matrigel were also correlated to MMP-9 expression. MMP-9 inhibitors suppressed these cell behaviors, indicating that MMP-9 activity is required for these phenomena. Flow cytometrical and immunohistochemical analyses showed that MMP-9 was associated with the cell surface and condensed on the lamellipodia. The following results indicated the possibility of cell surface heparan sulfate proteoglcan as an MMP-9 acceptor. 1) It was confirmed that both latent and active forms of MMP-9 had high affinity to heparin. 2) Treatments of the cells with heparitinase or heparin resulted in release of MMP-9 from the cell surface. Furthermore cell migration and invasion were inhibited by these treatments. 3) Co-localization of MMP-9 and heparan sulfate on lamellipodia was immunohistochemically detected. Taken together, it was strongly suggested that cell surface heparan sulfate proteoglycan acts as the acceptor of MMP-9 and locates MMP-9 on lamellipodia where the enzyme may actively degrade extracellular matrix to move the cell. It may mediate invasion and metastasis of tumor cells.

Participation of cell surface heparan sulfate proteoglycans in regulation of MMP-activation and localization does not merely explain behaviors of tumor cells, and it may be indicating their common function on interaction between cell and microenvironment.

Keywords: cell surface heparan sulfate proteoglycan, syndecan-2, matrix metalloproteinase, tumor cells, invasion and metastasis