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Suppression of EMMPRIN-mediated Tumor Cell Migration by Syndecan-1

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Objective: EMMPRIN (extracellular matrix metalloproteinase inducer) is a membrane-bound glycoprotein with two extracellular loop domains. We have previously revealed that EMMPRIN enhances tumor cell migration through an active site in the second loop domain (termed EM9). On the other hand, EMMPRIN has been reported to bind to various functional molecules through the extracellular loop domains, which is closely associated with the physiological and pathological functions of EMMPRIN. However, the molecular mechanism of EMMPRIN-mediated tumor cell migration is still unclear. In the present study, we examined the association of Syndecan-1, a membrane-bound heparan sulfate proteoglycan, with EMMPRIN-mediated tumor cell migration in human uterine cervical carcinoma SKG-II cells.

Methods: The expression of EMMPRIN and Syndecan-1, and their interaction on the cell surface of SKG-II cells was investigated by immunocytochemical, Western blot, and co-immunoprecipitation analyses. Cell migration was measured by scratch-wound assay.

Results: EMMPRIN and Syndecan-1 were co-localized on the cell surface of SKG-II cells. Syndecan-1 was found to form a complex with EMMPRIN through its heparan sulfate, whereas the enzymic deletion of N-glycosylation in EMMPRIN did not alter the interaction between Syndecan-1 and EMMPRIN. A synthetic EM9 peptide was found to interfere with the EMMPRIN-Syndecan-1 interaction. In addition, the deletion of heparan sulfate in Syndecan-1 by heparinase, derived from a Gram-negative bacteria, was found to facilitate SKG-II cell migration. Furthermore, the cell migration of SKG-II cells was dose-dependently augmented by administering an antibody against the EM9 peptide.

Conclusion: These results provide novel evidence that Syndecan-1 negatively regulates EMMPRIN-mediated tumor cell migration by a heterogeneous complex formation in that heparan sulfate of Syndecan-1 interacts with the EM9 region in the second loop domain of EMMPRIN.

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Release of emmprin as glycocalyceal bodies

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Objective: Emmprin is involved in tumorigenesis via stimulating production of matrix metalloproteinases (MMP), hyaluronan, and vascular endothelial growth factor (VEGF) by stromal fibroblasts and tumor cells. In human tumors, peritumoral fibroblasts that are not in direct contact with emmprin-expressing tumor cells are frequently stimulated to produce MMP. Here we investigated the mechanisms of emmprin release [1].

Methods and Results: Conditioned medium (CM) of human epithelioid sarcoma cell line FU-EPS-1 stimulated MMP-2 production by dermal fibroblasts, and this stimulation was inhibited by anti-emmprin antibody. Fractionation of CM by ultracentrifugation revealed selected presence of highly glycosylated form of emmprin (60 kDa) in the small vesicle fraction (glycocalyceal body fraction) compared with the larger vesicle fraction. This 60 kDa form of emmprin reacted with antibodies raised against N- and C-terminal portions of emmprin, indicative of the release of full length emmprin. Localization of emmprin on glycocalyceal bodies was also identified by immunoelectron-microscopical techniques. Biochemical and biological characteristics of emmprin-positive glycocalyceal bodies were studied.

Conclusions: Our results indicate that emmprin is released as glycocalyceal bodies from tumor cells and may stimulate peritumoral fibroblasts.

REFERENCES