Testican 2 Abrogates Inhibition of Membrane-Type Matrix Metalloproteinases by Other Testican Family Proteins.

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Testican family protein are putative extracellular heparan/chondroitin sulfate proteoglycans of unknown function. Recently we identified N-Tes, which is a product of testican 3 splicing variant gene, as an inhibitor of membrane-type matrix metalloproteinases (MT-MMPs). The inhibitory function is common among testican family members except for testican 2, which was shown to uniquely abolish inhibition of MT1-MMP- or MT3-MMP-mediated pro-matrix metalloproteinase-2 activation by other testican family members. Testican 2 inactivates N-Tes by binding to the C-terminal extracellular calcium-binding (EC) domain of N-Tes through its N-terminal unique domain as demonstrated by co-immunoprecipitation analysis, and thus testican 2 was unable to inactivate an N-Tes deletion mutant lacking the EC domain (N-Tes-Δ122). Migration of U251 cells on collagen which was dependent on MT1-MMP activity under serum-free condition, was inhibited by N-Tes or N-Tes-Δ122 deposited on collagen. Testican 2 was not incorporated into collagen by itself, and was deposited only in the presence of N-Tes, suggesting that testican 2 bounds to N-Tes deposited on collagen. Binding of testican 2 to N-Tes deposited on collagen allowed migration of cells expressing MT1-MMP.
Unlike wild-type N-Tes, N-Tes-Δ122 did not bind to testican 2, and thus expression of testican 2 did not recover cell migration blocked by N-Tes-Δ122. *In situ* hybridization showed that neurons are major source of all testican family members in the normal brain. The quantitative reverse transcription-polymerase chain reaction analysis demonstrated that all testican family members are expressed prominently in normal brain, and their expression levels decrease as tumor grade increases. The expression level of testican 2 was the highest among testican family members regardless of histological grade of astrocytic tumors. These results suggest that abundant distribution of testican 2 may contribute to glioma invasion by inactivating other testican family members including N-Tes, which all inhibit MT-MMPs. We propose that N-Tes-Δ122, which is resistant to testican 2, may have therapeutic potential as a barrier against glioma invasion.

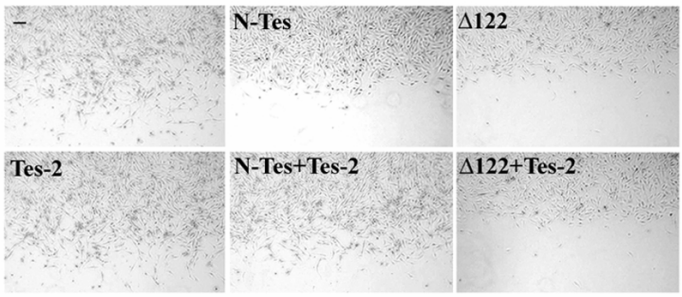

Fig. 1 Effects of testican on migration of U251 glioma cells expressing MT1-MMP. U251 cells transfected with MT1-MMP and indicated plasmids were subjected to wound-induced migration assay on plates coated with collagen.

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