Latent TGF-β Binding Proteins: Adhesive Functions and Matrix Association of LTBP-2 and Potential Functions of LTBP-1 and LTBP-3 in Mesothelioma

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Piia Vehviläinen, M.Sci., defended her Ph.D. thesis in Helsinki on May 7th, 2010. Her supervisor was Professor Jorma Keski-Oja, and her opponent Docent Sirkku Peltonen from the Department of Dermatology, University of Turku, Finland. This thesis can be found at http://urn.fi/URN:ISBN:978-952-10-6189-9.

Latent transforming growth factor-beta (TGF-beta)-binding proteins (LTBPs)-1, -3 and -4 are ECM components whose major function is to enhance the secretion and matrix targeting of the multifunctional cytokine TGF-beta. LTBP-2 does not bind to latent TGF-beta but has been suggested to function as a structural component of ECM microfibrils. In the current study, we analyzed the possible adhesive functions of LTBP-2 and characterized the kinetics and regulation of its secretion and association with the ECM. We also explored the role of TGF-beta-binding LTBPs in endothelial cells activated to recreate angiogenesis, as well as in malignant mesothelioma.

We found that, unlike most adherent cells, several melanoma cell lines efficiently adhered to purified recombinant LTBP-2. Further characterization revealed that this adhesion was mediated by alpha3beta1 and alpha6beta1 integrins. Heparin inhibited melanoma cell adhesion, suggesting a role for heparan sulfate proteoglycans. LTBP-2 was further identified as a haptotactic substrate for melanoma cell migration. We used cultured human embryonic lung fibroblasts to analyze the temporal and spatial association of LTBP-2 with the ECM. We found that LTBP-2 was only efficiently assembled into the ECM in confluent cultures and following the deposition of fibronectin (FN) and fibrillin-1. In early, subconfluent cultures it primarily remained in soluble form following its secretion. LTBP-2 colocalized transiently with FN and fibrillin-1. Silencing fibrillin-1 expression using lentiviral shRNAs profoundly impaired the deposition of LTBP-2, indicating that the association of LTBP-2 with the ECM depends on the presence of a pre-formed fibrillin-1 network. In view of the established role of TGF-beta as a regulator of angiogenesis, we induced morphological activation of endothelial cells using phorbol 12-myristate 13-acetate (PMA) and followed the fate of LTBP-1 in the endothelial ECM. We observed profound proteolytic processing of LTBP-1 and the release of latent TGF-beta complexes from the ECM. LTBP-1 processing was accompanied by increased activation of MT-MMPs and specific upregulation of MT1-MMP. That MT1-MMP plays a key role in the proteolysis of LTBP-1 was confirmed by suppressing its expression using lentivirally-induced short-hairpin RNAs, as well as various matrix metalloproteinase inhibitors. TGF-beta can promote tumorigenesis of malignant mesothelioma (MM), which is an aggressive tumor of the pleura. The prognosis for MM patients is typically poor. We analyzed TGF-beta activity in a panel of MM tumors through immunohistochemical staining of phosphorylated Smad-2 (P-Smad2). While LTBP-1 immunoreactivity was abundant in the stroma, tumor cells were found to contain significant amounts of P-Smad2. Moreover, there was a negative correlation between levels of LTBP-1 and P-Smad2 staining. In addition, strong P-Smad2 immunoreactivity correlated with shorter patient survival. mRNA expression analysis revealed that TGF-beta1 was the most highly expressed TGF-beta isoform in both normal human pleura and MM tissue. LTBP-1

Piia Vehviläinen completed her Ph.D. studies in Helsinki in May 2010. The defendant (middle) flanked by two dermatologists – her supervisor Jorma Keski-Oja (left) and opponent Sirkku Peltonen (right).

and LTBP-3 were also strongly expressed: while LTBP-1 was the predominant isoform in established MM cell lines, LTBP-3 expression was high in control cells. Suppression of LTBP-3 expression using siRNAs increased TGF-beta activity in MM cell lines, a response that was accompanied by decreased cellular proliferation. Our results suggest that decreased expression of LTBP-3 in MM may alter the targeting of TGF-beta to the ECM, leading to its increased activation.