Alternative splicing and processing of latent TGF-beta binding protein-1 (LTBP-1) in dermis

Zenso Isogai1, Akiko Ohno-Jinno2, Ken Watanabe1, Makoto Yamanaka1, Masahiko Yoneda2, Daniel B Rifkin4

1National Center for Geriatrics and Gerontology, 2Department of Ophthalmology, Aichi Medical University, 3Aichi Prefectural College of Nursing and Health, 4New York University School of Medicine

Latent TGF-beta binding protein-1 (LTBP-1) is a key molecule for extracellular regulation of TGF-beta. It is well known that TGF-beta is a potent regulator for extracellular matrix. LTBP-1 covalently binds to the propeptide of TGF-beta called latency associated protein, and LTBP-1 is a component of extracellular matrix. LTBP-1 interacts with fibrillin-1 and localizes to microfibrils in some tissues. LTBP-1 is composed of calcium binding EGF-like motifs, eight cysteine motifs and some uncharacterized motifs, such as the hinge region. The hinge region of LTBP-1 is essential for integrin alpha v beta 6 dependent activation of TGF-beta. A recombinant polypeptide of LTBP-1, containing the hinge region, interacts with fibronectin and fibronectin is required for integrin alpha v beta 6 dependent activation of TGF-beta. However, the hinge region can be alternatively spliced. Therefore, we focused on the unique alternative splicing variant called LTBP-1delta53 that lacks the hinge region.

RT-PCR analysis showed the expression of LTBP-1delta53 by human dermal fibroblasts. Both the long (LTBP-1L) and short (LTBP-1S) forms of LTBP-1 occurred as LTBP-1delta53 and non-spliced LTBP-1 in skin. Specific antibodies for LTBP-1delta53 and non-spliced LTBP-1 were generated using synthetic peptides. Using these antibodies, we demonstrated the secretion of LTBP-1delta53 and non-spliced LTBP-1 from skin fibroblasts. In dermis, LTBP-1 co-distributes with fibrillin-1 and versican but not with fibronectin.

In order to characterize the biochemical properties of LTBP-1delta53, recombinant LTBP-1 fragments representing LTBP-1delta53 and non-spliced LTBP-1 were designed, expressed and purified. Binding affinity to heparin is absent in LTBP-1delta53, however, susceptibility to plasmin is unchanged between LTBP-1delta53 and non-spliced LTBP-1.

An extraction study with 6M guanidine hydrochloride from mature skin showed the presence of macro aggregates consisting of the amino-terminus of LTBP-1. Moreover in the dermis, the amino-terminus of LTBP-1 containing the hinge region is cleaved from the rest of LTBP-1 containing the TGF-beta binding region. The macro aggregates, consisting of amino-termini of LTBP-1 are partially reducible, due to unpaired cysteine generated by the alternative splicing.

From these results we conclude that the unique alternative splicing and processing of LTBP-1 can be a critical modulator of TGF-beta activity in dermis. Moreover, ECM components in dermal microfibrils, such as fibrillin and versican, may affect TGF-beta activation through LTBP-1.