Abstract

The assembly of collagen VI microfibrils is a complex multi-step process in which proteolytic processing of the C-terminal globular region of the collagen VI α3 chain plays a major role. However, little is known about the mechanisms involved. One fragment, which is cleaved off during this process is the C5/Kunitz domain, the most C-terminal domain of the α3 chain. In its released form it is referred to as endotrophin and is thought to act as an adipokine to enhance tumor progression, fibrosis, inflammation and insulin resistance. Furthermore, the serum endotrophin level appears to be a useful biomarker to monitor the progression of several disorders such as chronic obstructive pulmonary disease, systemic sclerosis and kidney diseases. In this work, bone morphogenetic protein 1 (BMP-1) could be identified as the extracellular metalloproteinase responsible for endotrophin release. By mass spectrometry analysis the exact cleavage site within the α3 chain was identified. Immunoblot analysis revealed that only minor amounts of free endotrophin were detectable in tissue, indicating an only locally restricted activity of this adipokine. Moreover, a variety of larger endotrophin-containing fragments were present in various tissues and body fluids. Among these, a fragment spanning the C2-C5 domain is released by furin-like proprotein convertase cleavage. Using primary dermal fibroblast cultures and affinity purified antibodies against the N-terminal region and the C5 domain of the α3 chain, it could be shown that this proteolytic maturation occurs after secretion of collagen VI tetramers and during microfibril formation. Differential localization of N- and C-terminal parts of the collagen VI α3 chain revealed a unique deposition of cleavage products in tissue and cell culture pointing to an independent role of these fragments different from the functions described for collagen VI microfibrils. A strong intracellular signal with the C5 antibody in fibroblast cultures implicates an internalization of endotrophin and/or C5-containing fragments. Re-uptake of endotrophin and C5-containing fragments in RPE-1 cells, the classical cell system to study endocytosis, could only be shown for the 70 kDa C2-C5 fragment. The uptake is mediated via clathrin-coated vesicles, whereby the C2-C5 fragment is eventually degraded in lysosomes. This detailed information on the proteolytic processing of the collagen VI α3 chain provides a solid basis for elucidating the function of endotrophin and larger C5-containing fragments, to refine their use as biomarkers and study their contribution to collagen VI associated myopathies.