Regulation of Proteoglycan Synthesis by tumor necrosis factor-α (TNF-α) in Cultured Vascular Smooth Muscle Cells

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Proteoglycans are macromolecules that influence cellular behavior through interacting with extracellular matrix and growth factors/cytokines. On the other hand, proteoglycan synthesis is regulated by growth factors/cytokines. TNF-α is a cytokine that is involved in the progression of atherosclerosis. In the present study, we investigated the effects of TNF-α on the synthesis of proteoglycans using bovine aortic smooth muscle cells in a culture system. It was observed that TNF-α increased the accumulation of proteoglycan in the conditioned medium of the cells particularly when the cell density was high in dose- and time-dependent manners. The proteoglycans in the conditioned medium were separated into the high M_r subclass and the low M_r subclass by Sepharose CL-2B molecular sieve chromatography; TNF-α selectively increased the high M_r subclass with a decrease in the hydrodymanic size. After further purification by DEAE-Sephacel ion exchange chromatography, the high M_r subclass were identified as a large chondroitin sulfate proteoglycan versican by Sepharose CL-6B chromatography, SDS-PAGE, and Western blot analysis; the length of the chondroitin sulfate chains was reduced by TNF-α from M_r ~45,000 to M_r ~31,000. The disaccharide units of the chondroitin sulfate chains were detected as GlcA-GalNAc, GlcA-GalNAc(4S), GlcA-GalNAc(6S), GlcA-GalNAc(4S,6S), and GlcA(2S)-GalNAc(6S) in fluorophore-assisted carbohydrate electrophoresis; TNF-α particularly increased the disaccharide unit of GlcA-GalNAc(4S). The present results clearly indicate that TNF-α not only induces the synthesis of versican core protein but also influences the length and disaccharide composition of the chondroitin sulfate chains of versican in vascular smooth muscle cells.