



In vivo function of versican/PG-M: analysis of knockin mice

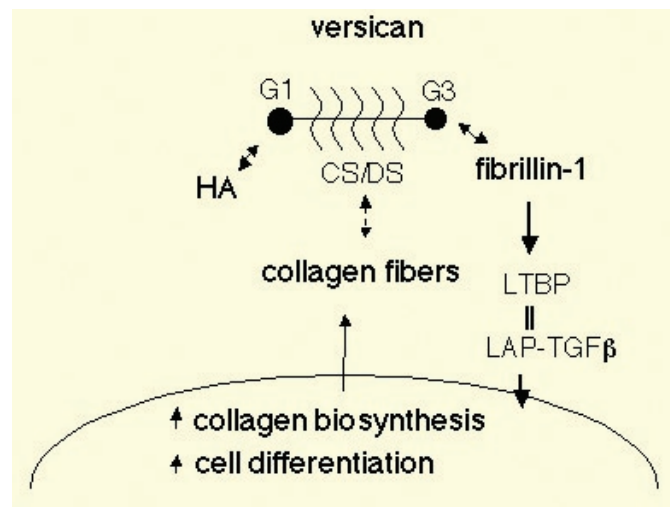
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Hideto Watanabe received M.D. in Kanazawa University School of Medicine in 1985, and Ph.D. in Pathology in 1989. In the middle two years as a graduate student, he worked with Professor Yutaka Nagai at Tokyo Medical and Dental University on biochemistry of collagens and serine proteinases that degrade the extracellular matrix molecules. In

following three years, while trained as a surgical pathologist at Kanazawa University Hospital, he continued to study on matrix metalloproteinases as an assistant professor at Cancer Research Institute. From July 1992 to January 2000, he worked with Dr. Yoshihiko Yamada at National Institute of Dental and Craniofacial Research, NIH. His research focused on molecular biology and genetics of aggrecan and link protein. There, he met Professor Koji Kimata when he was a Fogarty Scholar at NIH. He is an associate professor at Prof. Kimata's laboratory.



The regulation of cell behavior by the extracellular matrix (ECM) includes the incorporation of cytokines and growth factors into the ECM through their specific binding to ECM molecules. For instance, TGFβ is secreted as a latency-associated peptide (LAP)-TGFβ complex, which binds other macromolecules termed latent TGFβ-binding proteins (LTBPs). LTBPs interact with other ECM molecules such as fibrillin-1. Thus,

the supramolecular structure by matrix assembly is necessary for the deposition and appropriate signaling of cytokines and growth factors. Versican/PG-M is a large chondroitin sulfate proteoglycan (CSPG) of the ECM, which contains a core protein of approximately 550 kDa and up to twenty-three chondroitin sulfate (CS) chains, and is characterized by distinct expression patterns. It is transiently expressed in embryonic tissues such

as the heart, cartilage primordium, and nervous systems, and regulates cell adhesion, migration, proliferation and differentiation. Transiently expressed versican functions near the cell surface without being incorporated into the ECM. In some adult tissues such as the blood vessels and brain, versican is constitutively expressed and serves as a structural macromolecule of the ECM in the form of a proteoglycan aggregate.

The incorporation of versican in the ECM requires specific binding of the core protein to other molecules. The core protein consists of two globular domains, G1 and G3, and two CS-attachment domains, CS α and CS β . The N-terminal globular domain comprises three looped subdomains, A, B and B'. The B-B' segment interacts with HA, which is enhanced by the A subdomain. The G1 domain binds a member of the link protein family, termed hyaluronan and proteoglycan link proteins (HAPLNs), and the stability of the proteoglycan aggregate largely depends on the specific interaction of these three molecules, or the presence of HAPLNs. The C-terminal G3 domain interacts with other ECM molecules, including tenascins, fibulin-1, -2, and fibrillin-1; thus, versican is incorporated into the ECM at both termini with these globular domains, and CS chains attached to the middle of CS domains function there.

The developing heart exhibits dynamic expression patterns of versican, suggesting its role in chamber specification, growth and fusion of atrioventricular septa, and formation of the endocardial cushion.

As suggested by these observations, versican-null generated by gene-trap techniques die at embryonic day 10.5 (E10.5), exhibiting severe heart defects (Mjaatvedt et al., 1998). The future right ventricle and conus/truncus of the single heart tube fail to form, and the endocardial cushion in the arterio-ventricular and conus/truncus regions are absent. However, the role of versican in heart development is not fully understood, and death at E10.5 prevents the analysis of other tissues where versican is expressed in the late embryonic stages.

Recently we generated knock-in mice *Cspg2* ^{$\Delta 3/\Delta 3$} whose versican lacks the A subdomain of an N-terminal globular domain, G1. The homozygotes (*Cspg2* ^{$\Delta 3/\Delta 3$}) were small with dilated heart, and died by E18.5. Histologically, all the chambers appeared large, and heart wall was thin with increased deposition of collagen fibers. *Cspg2* ^{$\Delta 3/\Delta 3$} aortic wall was thin with decreased deposition of versican/PG-M. By immunostaining with smooth muscle α -actin, smooth muscle cells appeared immature in *Cspg2* ^{$\Delta 3/\Delta 3$} heart and aorta. *Cspg2* ^{$\Delta 3/\Delta 3$} skin showed loose dermis with less fibers and capillaries. Analysis using embryonic fibroblasts displayed impaired collagen fiber formation, reduced Smad2/3 nuclear translocation, and decreased deposition of latency-associated peptide (LAP)-TGF β complex in *Cspg2* ^{$\Delta 3/\Delta 3$} fibroblasts. In the ECM of their culture, fibrillin-1 was decreased together with versican. These observations suggest that versican, interacting with fibrillin-1, determines the local concentration of TGF β in the ECM and regulates its signal transduction.

Keywords: versican, PG-M, TGF β , cardiovascular systems, differentiation