Fibulin-1 integrates subendothelial extracellular matrices and contributes to anatomical closure of the ductus arteriosus.

Satoko Ito\textsuperscript{1,2}, Utako Yokoyama\textsuperscript{1,2}, Taichi Nakakoji\textsuperscript{2}, Yuko Kato\textsuperscript{1}, Junichi Saito\textsuperscript{1,2}, Naoki Nicho\textsuperscript{2}, Masuda Munetaka\textsuperscript{3}, Toshihide Asou\textsuperscript{4}, Yoshihiro Ishikawa\textsuperscript{2}

\textsuperscript{1}Department of Physiology, Tokyo Medical University, \textsuperscript{2}Cardiovascular Research Institute, Yokohama City University, \textsuperscript{3}Department of Surgery, Yokohama City University, \textsuperscript{4}Department of Cardiovascular Surgery, Kanagawa Children’s Medical Center

**Objective:** COX inhibitors targeting smooth muscle cell (SMC) contraction represent the only pharmacological treatment for patent ductus arteriosus (PDA), but >30% patients are resistant to the current therapies. Intimal thickening (IT), occurs in the subendothelial region of DA to bring anatomical DA closure. We investigated the role of fibulin-1 in DA anatomical closure to seek a new IT-inducing pharmacological therapy.

**Approaches and results:** Microarray analysis demonstrated that fibulin-1 was the most up-regulated gene by stimulation of EP4 in DA-SMCs. EP4-induced fibulin-1 expression was mediated through the phospholipase C-protein kinase C-noncanonical nuclear factor-kappa B pathway. We performed FACS analysis and found that fibulin-1 binding protein versican was derived from DA-endothelial cells. Immunofluorescence demonstrated that fibulin-1 and versican V0/V1 were co-expressed at the IT of wild-type DA. In the DA of EP4-deficient mouse (Ptger4\textsuperscript{-/-}), fibulin-1 was largely attenuated and showed PDA. All of fibulin-1-deficient mice exhibited PDA with hypoplastic IT, and fibulin-1 protein administration restored IT formation of Ptger4\textsuperscript{-/-}. Furthermore, 30% of versican deleted mice lacking a hyaluronan binding site displayed PDA.

**Conclusions:** Fibulin-1 contributes to DA closure by forming an environment favoring directional SMC migration toward the subendothelial region in combination with versican and hyaluronan. Targeting fibulin-1 upregulation may provide the basis for therapeutic strategies for inducing anatomical DA closure.