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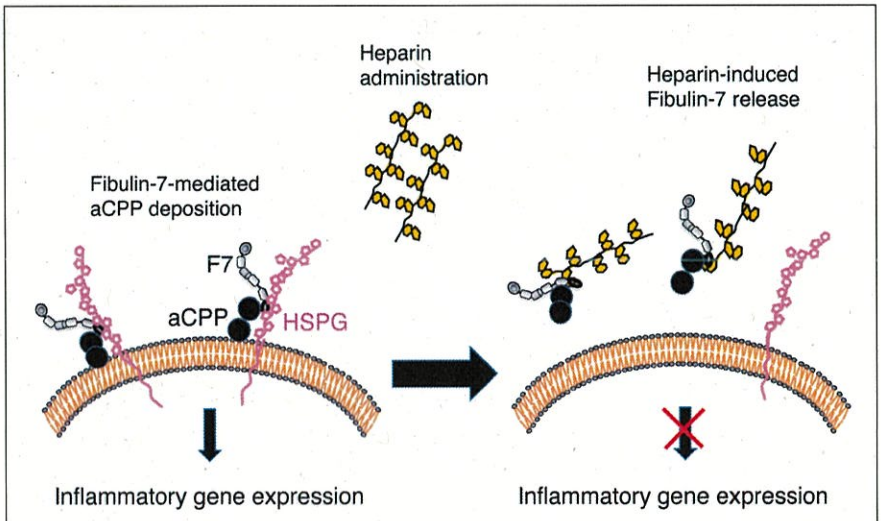
Grant Title: Intervention of renal calcification focusing on heparin-binding matrix fibulin-7

(a) Abstract

1) Aim: Ectopic calcification occurs during development of chronic kidney disease and has a negative impact on long-term prognosis. The precise molecular mechanism and prevention strategies, however, are not established. Fibulin-7 (Fbln7) is a matricellular protein structurally similar to elastogenic short fibulins, shown to bind dental mesenchymal cells and heparin. The aim of this study is to establish a basis of intervention strategy against renal calcification, focusing on the interaction between fibulin-7 and heparin on renal tubular epithelium.

2) Methods: A series of deletion mutants of fibulin-7 were generated and expressed in cell lines (CHO-K1, HEK293) and recombinant proteins were prepared from conditioned media of each cell line. Heparin binding assays and artificial calcium phosphate particle (aCPP) binding assays were performed using full-length and various deletion mutants. Evaluation of renal calcification, renal function, and transcriptional analysis were performed using wild-type and *Fbln7*-deficient (*Fbln7*KO) mice on normal diet or high phosphate diet.

3) Results: In vitro analysis revealed that fibulin-7 bound heparin at the N-terminal coiled-coil domain. In *Fbln7*-expressing CHO-K1 cells, exogenous heparin increased the release of fibulin-7 into conditioned media in a dose-dependent manner. This heparin-induced fibulin-7 release was abrogated in CHO-745 cells lacking heparan sulfate proteoglycan or in CHO-K1 cells expressing the *Fbln7* mutant lacking the N-terminal coiled-coil domain, suggesting that fibulin-7 was tethered to pericellular matrix via this domain. Interestingly, *Fbln7*KOmice were protected from renal tubular calcification induced by high phosphate diet. Mechanistically, fibulin-7 bound artificial calcium phosphate particles (aCPP) implicated in calcification and renal inflammation. The binding was significantly decreased in *Fbln7KO* primary kidney cells relative to wild-type cells. On the other hand, overexpression of *Fbln7* increased binding to aCPP. Addition of heparin reduced binding between aCPP and wild-type cells to the level of *Fbln7*KO cells.



Schematic presentation of the contribution of Fibulin-7 (F7) in renal cell calcification under high-phosphate diet. Inhibitory action of heparin on F7 is shown. The interaction between F7, artificial calcium phosphate (aCPP), and heparan sulfate proteoglycan (HSPG) occurs at pericellular space and exogenous heparin releases F7 into media by binding to the N-terminal portion of F7.

Our study suggests that fibulin-7 is a local mediator of calcium deposition and that releasing fibulin-7 from the cell surface by heparin/heparin derivatives or fibulin-7 inhibitory antibodies may provide a novel strategy to prevent ectopic calcification in vivo.