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Functional Analysis of a Molecule that Promotes Hypertrophy-Induced Fibrosis of the Heart and Kidney for Developing a New Fibrotic Treatment Method

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Summary

Salt is essential for a delicious diet that enriches people's minds, as well as for the maintenance of human life. On the other hand, excessive salt intake is one of the major causes of hypertension. Tissues whose function is particularly impaired by the development of hypertension are the heart and kidneys. The main cause of this decline in function is fibrosis in each tissue. Fibrosis is the excessive production of extracellular matrix proteins such as collagen, which greatly reduces tissue function by hardening the tissue.

Hypertension in the heart causes hypertrophy of cardiac muscle cells, which release various factors that activate surrounding fibroblasts. By these factors, fibroblasts differentiate into 'myofibroblast' and produce excessive amounts of extracellular matrix proteins such as collagen, leading to progression of cardiac fibrosis. Hypertensive patients, on the other hand, are known to be at increased risk for chronic kidney disease. Chronic kidney disease, regardless of its cause, is almost always accompanied by fibrosis of the kidneys. That is, hypertension also induces tissue fibrosis in the kidney.

Therefore, if the progress of fibrosis can be suppressed, it is considered that the functional depression of the heart and kidney at the time of hypertension can be suppressed. However, there is still no definitive method for controlling fibrosis, and it is desired to establish an epoch-making therapy and a therapeutic agent for fibrosis. In this context, we have identified FPF (Fibrosis promoting factor) as a new molecule involved in cardiac fibrosis. In other words, by using a model of myocardial infarction (left coronary artery stenosis) mouse with fibrosis, we have found

(1) FPF is rarely expressed in the normal heart and kidney, but is markedly increased in the fibrosis of the heart and kidney.
(2) Knockdown of FPF significantly reduces the production of extracellular matrix proteins such as collagen in myofibroblasts isolated from mouse hearts subjected to myocardial infarction model.

These results suggest that FPF is an important molecule that promotes cardiac and renal fibrosis.

In this study, we examined whether FPF is involved in the fibrosis of the heart and kidney in hypertensive mice and aimed to establish the foundation for the development of a new fibrotic therapy targeting FPF.

At first, the change of the expression level of FPF in the heart of the hypertensive model mouse was measured using the real time RT-PCR method. The results showed that FPF expression increased with cardiac hypertrophy. We further investigated whether FPF expression is increased during renal fibrosis using a model of renal fibrosis

using unilateral ureteral ligation (UUO). As in the case of the heart, a marked increase in FPF expression was observed during kidney fibrosis.

Next, we examined cells expressing FPF in fibrotic mouse heart and kidney by using in situ hybridization method. As a result, the FPF expression was found only in the fibrotic area, and the expression was not detected in the non-fibrotic area. Furthermore, the signal of FPF was observed only in cells expressing αSMA or Periostin, marker molecules of myofibroblasts, indicating that FPF is specifically expressed in myofibroblasts.

In order to examine the involvement of FPF in the fibrotic disease in vivo, we set out to make the knockout mouse of FPF using the GONAD method (Ohtsuka M et al., Genome Biol., 2018). Mouse FPF consists of approximately 500 amino acids, and we designed a stop codon approximately 30 amino acids from the start codon of FPF and performed genome editing using the Crispr-Cas9 system. We succeeded in obtaining hetero mice with mutations in the target genome. These mice were mated with WT mice to ensure that they were on the germline, and then mated with heterozygous offspring to obtain FPF KO mice. Indeed, when cardiac myofibroblasts were isolated from these mice and Western blotting was performed using antibodies to FPF, the desired band disappeared, indicating that FPF protein was really knocked-out. We are now breeding the KO mice. We plan to compare the fibrosis status of WT and KO mice by treating them with a hypertension model.