論文の内容の要旨

論文題目 **Cytoglobin, a novel globin, plays an anti-fibrotic role in the kidney.**

和訳:新規グロビン蛋白、サイトグロビンは腎臓において抗線維化作用を有する

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内科学専攻

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Recently two novel members of the globin superfamily, neuroglobin (Ngb) and cytoglobin (Cygb), were discovered. In contrast to conventional globins such as hemoglobin (Hb) and myoglobin (Mb), Ngb and Cygb display a functionally-relevant hexa-coordinate heme iron atom. While Ngb is predominantly expressed in nerve cells, Cygb is expressed in fibroblast cell lineage distributed in various organs. However, its function remains unknown.

In order to study the expression of Cygb in the kidney of rats, we made rabbit polyclonal antibodies against rat Cygb. Immunohistochemical staining with these antibodies

showed the expression of Cygb in interstitial fibroblasts of the normal kidney and the remnant kidney (RK) model, a model of renal fibrosis. Immunohistochemical analysis showed the up-regulation of Cygb in the interstitial cells in RK. Expression levels of Cygb in RK rats were significantly increased as the disease progressed at both the mRNA and protein levels. The association of Cygb up-regulation with fibrosis was confirmed by using immunohistochemistry for the detection of collagen I and collagen IV. Our morphometric analysis showed that up-regulation of Cygb was associated with an increase in collagen I and IV in the tubulointerstitium of RK. In addition, we observed the expression of Cygb was adjacent to collagen IV deposition areas, suggesting that Cygb expression was correlated with collagen synthesis in interstitial cells in RK.

To investigate the biological role of Cygb *in vivo*, we established a new transgenic (Tg) rat which overexpressed rat Cygb under the control of a ubiquitous CAGGS promoter. Cygb was expressed in various organs in these rats, including brain, heart, liver and kidney. To investigate the functional consequence of Cygb up-regulation, we induced RK in these animals. As a result, deterioration of renal function over 9 weeks as estimated by serum creatinine levels was ameliorated in Cygb-Tg rats with RK compared with wild type rats with RK. The amount of proteinuria was also less in Cygb-Tg rats with RK than in wild type animals. Evaluation of fibrosis of RK by morphometric analysis of collagen I and IV

accumulation showed that transgenic overexpression of Cygb ameliorated fibrosis of the kidney. Protection against fibrosis in Cygb-Tg rats was confirmed by Masson's trichrome staining.

To clarify an anti-oxidative role of Cygb, we transfected kidney fibroblast cell line NRK49F to overexpress Cygb. When Cygb-overexpressing NRK49F cells were treated with hydrogen peroxide, they displayed increased reactive oxygen species (ROS) scavenging activity compared with cells expressing a mock-vector.

In order to investigate the mechanism of amelioration of fibrosis by Cygb *in vivo*, we used nitrotyrosine as a marker of oxidative stress. Immunohistochemical analysis revealed a decrease in nitrotyrosine accumulation in tubules in RK in Cygb-Tg rats. Urinary 8OHdG as another marker of oxidative stress in Tg rats was significantly reduced compared with wild type animals.

To further clarify the mechanism by which renal fibrosis is ameliorated in Cygb-Tg rats, we established primary cultured fibroblasts from the kidneys of wild type and Cygb-Tg rats. Cygb-Tg rat-derived fibroblasts showed overexpression of Cygb at both the mRNA and protein levels compared with cells derived from wild type animals. Synthesis of collagen I was reduced in fibroblasts from Cygb-Tg rats in both mRNA (73.8%) and protein levels (43.5%) as compared to those in cells from wild type rats. We also established stable

transfectants in HEK293T cells in which Cygb can be overexpressed in an inducible manner. The amount of collagen I production measured by ELISA was also reduced in these cells when Cygb expression was induced.

We engineered mutant cells with impaired ability to bind ligands at the heme of Cygb to test whether the heme component of Cygb might be the main contributor to radical scavenging. In contrast to cells overexpressing intact Cygb, the amount of collagen I synthesis by kidney fibroblasts expressing mutated Cygb did not differ from that by control cells.

In conclusion, we demonstrated for the first time that Cygb plays a protective role against fibrotic changes in the kidney both *in vitro* and *in vivo* via the amelioration of oxidative stress. Cygb is a fascinating target for therapeutic approaches in a variety of fibrotic diseases, including chronic kidney disease.