Molecular profile of chloride channel specifically localized in macula densa cells

Hisato Sakamoto, [1] Takashi Matsuo, [1] Ichiro Naito, [2]Yasusi Nagaba, [1] and Shimizu Takeshi[1]
[1]Department of Internal Medicine, Kitasato University, [2]Shigei Medical Institute

In the kidney, tubuloglomerular feedback (TGF) plays a key role in maintenance of fluid and electrolyte balance of the body as well as in blood pressure regulation. Previous studies have demonstrated that the chloride channel specifically localized in macula densa cells might be involved in the regulation of this feedback system. However the precise molecular mechanism by which regulate its cellular function is still a matter of debate. Thus, to identify the relationship between its molecular structure and function is particularly important subject. In the present study, we have successfully isolated a specific MAb to stain immunologically the cells that were restrictively localized within the juxtaglomerular apparatus. This MAb is an anti-peptide antibody against 18 amino acid residue of extracellular loop between first transmembrane domain (D1) and second transmembrane domain (D2) of ClC-5 chloride channel, which is the conserved region among the channels belong to the ClC chloride channel family, and designated as MD12 MAb.

The intense immunoreactive staining with MD12 MAb was apparent in macula densa of most glomeruli in the mouse kidney cortex. However, it has to be carefully considered whether immunolocalization is in the afferent arterioles. In addition, confocal microscopic approach provided the finding that the immunoreactive protein was localized both in plasma membrane and cytoplasmic region in the cells. Further experiments are necessary to identify the precise localization at cell and subcellular level in the juxtaglomerular apparatus. In contrast, the expected molecular size of this immunoreactive protein was appeared to be around 50 kDa.

Subsequently, we have attempted to identify the gene of the immunoreactive protein with MD12MAb using the expression cloning strategy. The pre-made mouse kidney cDNA library was initially transfected into the COS-7 cells, which did not exhibit any detectable staining with MD12 MAb. Then, the cells expressing immunoreactive protein with MD12 MAb were isolated magnetically using magnetic beads coated with this antibody. Now, it is final step to identify the gene coding immunoreactive protein with MD12 MAb. Following the cloning of target gene, we would like to expand this project to confirm the putative roles of this novel protein in TGF system.