Objectives: MST3 (mammalian-Ste20-kinase 3) is a member of the Ste20 (sterile-20) kinase family. The MST3 was reported to inhibit cell migration by us. Recent studies indicate that other Ste20 kinases, such as proline-alanine-rich Ste20-related kinase (PASK) and oxidative stress-response protein 1 (OSR1) are conserved regulators of cell volume and ion transport and the regulation of pathogenesis of hypertension. The present study was to test whether MST3 is involved in the pathogenesis of hypertension.

Methods: The localization and expression level of MST3 were compared in the kidney of Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) by immunohistochemistry and western blotting. The level of MST3 was also measured before and after development of hypertension in SHR. Wild type MST3 (WT-MST3 mice) and constitutively activated MST3 transgenic mice (T178E-MST3 mice) were fed with normal-salt or high-salt diets. The urinary volume and urine ion excretion were compared between both groups of mice.

Results: The MST3 was mostly concentrated in principal cells of collecting duct in the inner medulla. However, MST3 markedly reduced in SHR. The difference expression level of MST3 already existed in prehypertensive SHR. In vivo, the urinary Na^+, K^+, Cl^- and water excretion was higher in T178E-MST3 mice than in WT-MST3 mice. Challenge mice with high-salt diets, the urinary Na^+, K^+, Cl^- and water excretion was significantly higher with *P value* <0.005.

Conclusion: It is the first report that MST3, a brand new gene in Ste20 kinases, may inhibit urinary sodium and water reabsorption in collecting duct of inner medulla.

Ste20 serine/threonine kinases in mammals regulate many fundamental cellular processes including cell-cycle control, apoptosis, development, cell growth, and cell

stress responses [1, 2]. Approximately 30 mammalian Ste20-related kinases have been identified [3]. Based on the degree of homology of their kinase domain, the mammalian Ste20 family is divided into the P21-activated kinase family (PAK) and the germinal center kinase (GCK) family. GCK is subdivided into GCK-I to GCK-VIII [4]. MST3/MST4/YSK1, belong to GCK-III, are all involved in signaling of cell polarity and important for cell migration (J Cell Biol. 2004, 164(7):1009-20. JBC Lu). Interference with MST4/YSK1 function perturbs cell migration, and invasion into type I collagen. Mutation of thr-174 in YSK1 abolishes collagen invasion and cell migration (J Cell Biol. 2004, 164(7):1009-20). It was also reported that MST3 regulates cellular migration and the regulation was altered by PTP-PEST activity. Mutation of trr-178 in MST3 inactivates the MST3 kinase activity *in vitro* and effects on migration *in vivo*. The threonine and its phosphorylation in activating loop of Ste20 family is highly conserved and required for kinase activity. These results indicate that they share the same upstream signal pathway.

In yeast, pMO25 was reported to regulate the function of the MST/Ste20 kinase homologue Ppk11 during cell morphogenesis. In mammalian, MO25 functions as a critical scaffolding subunit to stabilize the bound kinase and full kinase activation. MST3 was reported to bind to MO25 and stimulates MST3 kinase activity three- to four-fold. Proline-alanine-rich Ste20-related kinase (PASK; also known as SPAK) and oxidative stress-response protein 1 (OSR1), are members of GCK-VI subfamily. The thr-243 of SPAK and thr-185 of OSR1 in activating loop were mutated to glu to mimic phosphorylation. The mutant T243E-SPAK and T185E-OSR1 have also been reported to bind to MO25, resulting in approximately 100-fold activity increment. This interaction enhances PASK/OSR1 ability to phosphorylate their targets. The targets include Na-K-2Cl cotransporter 1 and 2 (NKCC1, NKCC2), Na-Cl cotransporter (NCC) and all K-Cl cotransporters (KCCs) [39]. However, the target of MST3 is still unknown.

Thr-185 in the activating loop of OSR1 kinase was reported to be phosphorylated by WNKs, which are another group of serine/threonine kinases. WNK are critical components of the signaling of blood pressure homeostasis in humans. Gain-of-function mutation of WNKs subsequently phosphorylate and activate PASK/OSR1. The PASK/OSR1 targets, NCC, NKCC1 and NKCC2, are phosphorylated and activated to increase sodium reabsorption in the kidney, resulting in hypertension. Thus, PASK and OSR1 are evolutionarily conserved regulators of epithelial fluid excretion, cell volume and ion transport and involved in the regulation of hypertension [2, 5-10]. Thr-178 of MST3 is conserved with thr-243 of SPAK and thr-185 of OSR1, which promotes kinase in active conformation. These results

prompt us to hypothesize that MST3 play a role in regulators of epithelial fluid excretion, cell volume and ion transport and blood pressure.

Increased sodium reabsorption in kidney tubulars is a genetic characteristic in the SHR [22-24]. SHR and the normotensive WKY rats have been widely used as an experimental model for sodium regulation in kidney [18]. To investigate whether MST3 regulate sodium homeostasis, the localization and expression of MST3 were compared in kidney between the normotensive WKY rats and SHR. The amount of ions in urine were analyzed and compared between wild type MST3 mice (WT-MST3) and transgenic mice expressing constitutively activated MST3 (T178E mice). Meanwhile, to investigate whether T178E transgenic mice are more potent to regulate sodium homeostasis, high NaCl diets were administrated and the amount of ions in urine were analyzed and compared again.