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Review

Molecular Biology of Blood Pressure Regulatory Genes

KAZUHISA TAKEUCHI

Division of Nephrology, Endocrinology, and Vascular Biology, Tohoku University Graduate School of Medicine, Sendai 980–8574

K. TAKEUCHI. *Molecular Biology of Blood Pressure Regulatory Genes*. Tohoku J. Exp. Med., 2001, **197**(1), 2002, 1–8 —— Blood pressure is determined by vascular resistance and circulating volume. Activation of vascular angiotensin II or thromboxane receptor is mostly involved in the former, and function of renal prostagalandin EP_3 receptor or thiazide-sensitive sodium-chloride co-transporter is also in the latter. We have cloned rat genes for these blood pressure regulatory factors, and studied their gene expression. Here we review the molecular biology of those genes, based on our observations. ———— Translational medicine; HFH-3; NRF-2; Sp-1; PPAR-γ, Gitelman's syndrome.

Hypertension is suggested to be caused by an abnormality of one blood pressure regulatory gene (monogenic hypertension) or by a combination of some unknown genetic disorders (polygenic essential hypertension) underlying the blood pressure regulation system. For instance, as monogenic hypertension, Liddle's syndrome (Takeuchi et al. 1989), dexamethasone-suppressible aldosteronism (Miura et al. 1986) and apparent mineralocorticoid syndrome (White et al. 1997) are caused by abnormality of epithelial type of sodium chloride (NaCl) channel (ENaC), 11β-hydroxylase/aldosterone synthases, and 11β-hydroxysteroid dehydrogenase, respectively (Lifton et al. 2001). With regard to essential hypertension, however, any definitive candidate genes have ever been identified, although many efforts to identify the genes have been conducted (Higaki et al. 2001). Angiotensinogene gene is one of the candidate genes for hypertension, and, interestingly, a mutation in its gene transcription region has been suggested to be related to hypertension (Ishigami et al. 1997), suggesting that transcription mechanism of hypertension candidate gene should be paid more attention. Here we review our observations in the molecular biology of angiotensin AT_1a receptor, thromboxane synthase/receptor, prostaglandin EP_3 receptor, and thiazide-sensitive sodium-chloride (Na-Cl) cotranporter in terms of blood pressure regulation.

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Address for reprints: Kazuhisa Takeuchi, M.D., Division of Nephrology, Endocrinology, and Vascular Biology, Tohoku University Graduate School of Medicine, Sendai 980–8574, Japan.

e-mail: hek293@mail.cc.tohoku.ac.jp

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Angiotensin AT_1 Recetpor

Angiotensin (A) II is a potent vasoconstrictor and stimulator of aldosterone synthesis leading to high blood pressure. These AII actions are mediated via its specific receptor, AT_1 subtype, which is linked to a calcium/inositol signal transduction pathway (Takeuchi et al. 1992) and a AP-1 mediated gene transcription system (Takeuchi et al. 1990). The rat cDNA (AT_1a) was cloned by Murphy et al. (1992), and the primary protein structure was revealed. The gene for AT_1a receptor has also been cloned, and its transcription function of its 5'-flanking region (FL) has been characterized (Takeuchi et al. 1993a). The transcription regulatory region of AT_1a receptor gene possesses TATA box, Sp-1 binding sites and GATA box and exerts potent transcription function in vascular smooth muscle (VSM) cells. AT_1a receptor gene was shown to be induced by glucocorticoid (Guo et al. 1995) and insulin (Nickenig et al. 1998), and suggested to be involved in the vascular hyperplasia caused by these substances. On the other hand, we have shown that interferon (IFN)-γ inhibited transcription of AT_1a receptor gene on the region containing gamma-interferon activation sites (GAS) dependent on a protein phosphorylation mechanism (Ikeda et al. 1999), suggesting mechanism of the IFN-γ inhibition against vascular smooth muscle proliferation. AT_1a receptor is thus suggested to mediate vascular homeostasis at variuos steps. Recently, angiotensin AT_1 receptor activation was shown to interact with insulin receptor signal transduction (Velloso et al. 1996), which would suppress glucose metabolism in peripheral tissues, probably causing insulin-resistance. An anti-DM compound troglitazone (one of thiazolidinediones) improves insulin-resistance, and not only ameliorates DM but also lower blood pressure in a group of DM patients (Ogihara et al. 1995), indicating a potential role of insulin resistance in pathogenesis of hypertension. Thiazolidiendiones are known to be ligands of peroxiosme proliferators-activated receptor (PPAR)-γ, a transcription factor that was initially shown to be involved in adipocyte differentiation. It has recently been shown that troglitazone inhibits VSM cell prolifertation and vascular neointimal formation after balloon injury (Hsueh and Law 2001), and dilates renal glomerular arterioles (Arima et al. 2002), suggesting a beneficial role of thiazolidinediones in vascular homeostasis. We examined the effect of PPAR-γ activation on gene expression of angiotensin AT_1a receptor in VSM cells. Interestingly, troglitazone inhibited AT_1a receptor mRNA expression in VSM cells, and it also inhibited transcription of AT_1a receptor gene dependent on PPAR-γ at a proximal Sp-1-binding site (Sugawara et al. 2001a). Other thiazolidinediones such as pioglitazone and rosiglitazone also inhibited the transcription (Sugawara et al. 2001b). Moreover, GST-pull down assays have indicated that PPAR-γ can directly interact with Sp-1 causing the transcription inhibition. It is thus suggested that anti-insulin resistance drug thiazolidinediones would ameliorate angiotensin II-induced vascular remodeling as well as improve glucose metabolism probably by activating PPAR-γ, although this molecular mechanism should be further translated in clinical subjects.

Thromboxane system: thromboxane synthase and receptor

Prostaglandin (PG) endoperoxide PGH_2 is a major metabolite of arachidonic acid (AA) formed by the cyclooxygenase (COX) pathway. PGH_2 is metabolized by thromboxane synthase (TXS) to thromboxane A_2 (TXA_2). PGH_2 and TXA_2 elicit platelet aggregation and vascular contraction by activating a specific thromboxane receptor (TP receptor). Salt-loading induced TP receptor mRNA expression in rat kidneys, and enhanced renal tubuloglromerular feed-back via TP receptor possibly leading to salt-sensitive hypertension (Welch et al. 1997). Thus, TXA_2 has been suggested to be involved

in atherosclerosis and hypertension.

cDNA for TXS was cloned from human lung (Ohashi et al. 1992) or rat kidney (Tsutsumi et al. 1997). Expression of TXS mRNA was shown to be induced in hydronephrotic and transplant kidneys, supporting the previously reported observation that TXA_2 synthesis was enhanced in these phathological conditions. Immunohistochemical studies of biopsy specimen show low levels of TXS expression in normal kidneys, but much enhanced expression in kidneys from renal transplant recipients (Berkes et al. 1997). We have also shown that significant expression of TXS in rat gonadal tissues though the functional significance remains unclear (Takahashi et al. 1995). Rat TXS gene has also been cloned, and rat TXS gene (Tbxas1) was shown to be located on chromosome 4q21-q22 (Takeuchi et al. 1997a). Rat TXS gene transcription function has been characterized, and it has been shown that nuclear factor (NF)-E2 binding site plays an important role in transcription of TXS gene (Ikeda et al. 2000). We first observed by mobility shift assays that the NF-E2 binding site was specifically bound by NF-E2 related factor 2 (NRF-2), and interestingly the transcription function of NRF-2 on TXS gene was suppressed by PPAR-γ activation via a protein-protein interaction mechanism. Actually, TXS mRNA expression was inhibited by PPAR-γ activators troglitazone in macrophages, suggesting that troglitazone would suppress TXA_2 biosysnthesis at the step of TXS gene transcription, and it could be an agent protecting against atherosclerosis.

TP receptor was first purified from human platelets by Ushikubi et al. (1989), and the isolation of its cDNA was accomplished (Hirata et al. 1991), followed by cloning of a rat cDNA (Abe et al. 1995). The amino acid sequence for the TP receptor shows seven hydrophobic domains. It is a member of the large family of seven transmembrane structural proteins characteristic of a G-protein-coupled receptor, and cloned TP receptor signal induces changes in inositol trisphosphate (IP_3) and (Ca^{2+}) messenger system. The mRNA for TP receptor can be detected by in situ hybridization in glomeruli, renal cortical arteries, and renal pelvis (Abe et al. 1995). Microscopic examination has localized TP receptor mRNA in glomeruli, vascular arterial smooth muscle cells, and transitional epithelium of renal pelvis. However, mRNA expression in tubules was minimal. Application of a more sensitive method of reverse transcription and polymerase chain reaction (RT-PCR) to rat microdissected nephron segments demonstrated mRNA exclusively in glomeruli, but not in tubules. However, immunohistochemical studies in the rat using antibody against TP receptor has localized it in renal tubules (Takahashi et al. 1996). Using an antibody against the carboxyl-terminal tail of rat TP receptor, we have showed that TP receptor is present in glomeruli, medullary thick ascending limb of Henle and in distal, but not in proximal tubules. On the other hand, using a polyclonal antibody against the TP receptor, there is abundant TP receptor expression in the brush border of proximal tubules (Bresnahan et al. 1996). Although the expression of TP receptor protein in proximal tubules is controversial, the expression at distal tubules is consistent.

The 5′-FL of the TP receptor gene that regulates its transcript has been cloned from both human (D'Angelo et al. 1995) and rat sources (Takahashi et al. 1998). It is GC-rich, lacks a TATA box-like sequence, but does possess various putative cis-acting elements such as NF-interleukin (IL)-6 (APRRE), GRE, AP-1, AP-2, Sp-1, and NF-κ-B sites, and the rat gene is mapped to chromosomes 7q11 (Takeuchi et al. 1996c). These 5′-FL has been shown to have active transcription activity. In VSM cells, both glucocorticoid, IL-6, and phorbor ester stimulated transcription of the 5′-FL of rat TP receptor gene. Thus TP receptor expression could be up-regulated by vasoactive agents that activate PKC or by cytokines generated

during inflammation. Since TP receptor stimulation of renal mesangial cells leads to cell contraction, and vascular TP receptor stimulation induces cell mitosis and cell hypertrophy, TP receptor-induction in an inflammatory condition would aggravate the tissue injuries. On the other hand, we have shown that TP receptor mRNA expression was down-regulated by thiazolidinediones (Sugawara et al. 2002). Similar to the transcription suppression of AT_1a receptor gene by thiazolidinediones (Sugawara et al. 2001a, b), TP receptor gene transcription was shown to be inhibited by these compounds. Moreover, molecular analysis has shown that the transcription suppression was due to the inhibition of transcription activity of Sp-1 by PPAR-γ activated by thiazolidinediones. Together with the transcription suppression of TXS gene, thiazolidinediones have been thus suggested to specifically inhibit TX system via activation of PPAR-γ.

Prostaglandin EP_3 receptor

Following the cloning of TP receptor, other types of PG receptor including PGE_2 receptor were cloned (Narumiya et al. 1999). Among isoforms of PGE_2 receptor, EP_3 subtype was shown to be abundantly expressed in kidney at the outer mellula region containing medullary thick ascending limb of Henle (mTAL) and collecting tubules (Takeuchi et al. 1993b, 1994a). Functional analysis has indicated that the cloned EP_3 receptor can antagonize against vasopressin V_2 receptor via inhibition of cyclic AMP formation or an increase in cytosolic Ca^{2+} (Takeuchi et al. 1994b, 1996a), providing a molecular background to the previously reported observation on the antagonism of PGE_2 against the vasopressin-sensitive water/sodium reabsorption via the cyclic AMP system (Grantham and Orloff 1968). Vasodepressor effect of PGE_2 has been thus suggested to be due to the activation of EP_3 receptor causing natriuresis. In EP_3 receptor, multiple isoforms have been identified and the gene cloning has shown that they are created by an alternative splicing mechanism (Narumiya et al. 2001). Any phathological change in expression of EP_3 receptor has not been found. Role of EP_3 receptor in human hypertension remains to be studied.

Thiazide-sensitive Na-Cl cotransporter gene

Renal tubular Na reabsorptioin is very important for electrolyte metabolism as well as blood pressure regulation. Na is rebasorpted at proximal tubules by around 70%; TAL by 15%; cortical distal tubules (distal convoluted tubule [DCT]) by 6–7%, and the following cortical collecting tubules (CCT) by 2–3% of filtered Na from glomeruli. At proximal tubules, Na is reabsorpted coupled with various substances such as proton, glucose, amino acids, etc. At TAL, DCT and CCT, however, there are their specific Na reabsorption mechanisms. Furosemide-sensitive Na-K-2Cl cotransporter (NKCC2) couples with both K channel (ROMK, in the luminal side) and Cl channel (CLCNKB, in the basolateral side) is the key molecule for NaCl reabsorption at TAL; thiazide-sensitive Na-Cl cotransporter (TSC) is the one at DCT, and epithelial type of Na channel (ENaC) is another at CCT (Hebert 1999). Disorders of these distal tubular Na reabsorption mechanisms have been shown to cause Bartter's syndrome, Gitelman's syndrome and Liddle's syndrome, respectively.

Gitelman's syndrome (Gitelman et al. 1966) is characterized by clinical findings such as dehydration and frequent tetany, and by laboratory findings such as hypokalemic metabolic alkalosis, secondary aldosteronism without high blood pressure, hypomagnesemia, and hypocalciuria. Since these findings are quite similar to those observed under the chronic ingestion of large amount of thiazide diuretics, this disorder believed to be caused by a defect in the thiazide-sensitive Na reabsorption at distal nephron. In 1996, mutations in TSC gene were identified in Gitelman's syndrome (Simon et al. 1996).

Moreover, a mutation found in TSC was shown to be well associated with familial Gitelman's syndrome (Takeuchi et al. 1996a), and later the mutation was also shown to cause loss of function of TSC (Taniyama et al. 2000). TSC has been thus shown to play an important role in electrolyte metabolism and blood pressure regulation. In a case of Gitelman's syndrome, however, we did not identified any mutation in TSC (Takeuchi et al. 1997b). It was suggested that the other genetic defect, for instance, a genetic defect in gene expression, could be involved in the case, and we studied the renal TSC gene expression mechanism.

TSC gene has been shown to be exclusively expressed at DCT. To address the molecular mechanism of this DCT-spscific localization of TSC gene expression, we studied transcription of rat TSC gene. The 5′-FL of rat TSC gene was cloned and its structure was shown to contain such sequences as TATA box, Sp-1 site, C/EBP sites, glucocorticoid-response element (GRE), cyclic AMP-response element (CRE), etc. (Taniyama et al. 2001a). Interestingly, we also identified a putative binding site for hepatocyte nuclear factor-3/folk head homologue-3, HFH-3 (Overdier et al. 1997), which was shown to be specifically expressed at renal cortical distal tubules. The 5′-FL of TSC gene possessed active transcription activity, and transgenic rats harboring the rat 5′-FL fused upstream of LacZ gene represented the right localization of β-galactosidase gene expression (derived from LacZ gene) to DCT. The cloned 5′-FL was thus shown to have DCT-specific transcription activity. We then further characterized the transcription mechanism with reference to HFH-3, and have indicated that an active HFH-3 site is present at -400/-387. A dominant-negative type of mutation of HFH-3 may possibly cause Gitelman's syndrome or some electrolyte/blood pressure disorders, and creation of its aminal model would provide an answer. Moreover, in response to dexamethazone and aldosterone, transcription activity of TSC gene was shown to be enhanced (Taniyama et al. 2000b). It could be interpreted that an increase in TSC gene expression may be involved at least in part in steroid-induced hypertension.

Beyond hypertension

Hypertension has recently been characterized as one of the findings of "common disease (multiple risk factor syndrome)" including diabetes mellitus (DM), obesity, hyperlipidemia etc., leading to atherosclerosis. Subjects with a mutation in PPAR-γ were shown to frequently present DM with insulin resistance and hypertension (Barroso et al. 1999), presumably indicating a common genetic background causing hypertension and sugar/lipid metabolic disorders. Especially, transcription factors involved in lipid metabolism such as sterol regulatory element binding protein (SREBP) (Ikeda et al. 2001) would be another target for understanding of a genetic background of common disease. For treatment of patients, understanding of molecular mechanism including gene transcription mechanism underlying the common disease will be more important. Susceptibility to hypertension or diabetes mellitus may possibly be dissected according to a genetic analysis of transcription factor, and the cost effective individual treatment would be realized under "tailor-made medicine". Gene transcription analysis has less been focussed in hypertension. To extend the scope of pathogenesis of hypertension, however, transcription mechanism of its candidate genes would be further explored.

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References

Abe, T., Takeuchi, K., Tsutsumi, E., Taniyama, Y. & Abe, K. (1995) Rat kidney thromboxane receptor: molecular cloning, signal transduction, and intrarenal expression localization. *J. Clin. Invest*., **96**, 657–664.

Arima, S., Kohagura, K., Takeuchi, K., Sugawara, A., Ikeda, Y., Abe, M., Omata, K. & Ito, S. (2002) Biphasic vasodilator action of troglitazone on the renal microcirculation. *J. Am. Soc. Nephrol*., **13**, 342–349.

Barroso, I., Gurnell, M., Crowley, V.E., Agostini, M., Schwabe, J.W., Soos, M.A., Maslen, G.L., Williams, T.D., Lewis, H., Schafer, A.J., Chatterjee, V.K. & O'Rahilly, S. (1999) Dominant negative mutations in human PPAR-γ associated with severe insulin resistance, diabetes mellitus and hypertension. *Nature*, **402**, 880–883.

Berkes, E.A., Croker, B.P., Barri, Y., Peterson, J.C., Wilcox, C.S. & Ramos, E.L. (1995) Thromboxane synthase expression co-localizes with infiltrating macrophages in renal allograft biopsies. *Kidney Int*., **48**, 1344–1346.

Bresnahan, B.A., Le Breton, G.C. & Lianos, E.A. (1996) Localization of authentic thromboxane A_2/prostaglandin H_2 receptor in the rat kidney. *Kidney Int*., **49**, 1207–1213.

D'Angelo, D.D., Davis, M.G., Houser, W.A., Eubank, J.J., Ritchie, M.E. & Dorn, G.W., II. (1995) Characterization of 5′ end of human thromboxane receptor gene. Organizational analysis and mapping of protein kinase C-responsive elements regulating expression in platelets. *Circ. Res*., **77**, 466–474.

Gitelman, H.J., Graham, J.B. & Welt, L.G. (1966) A new familial disorder characterized by hypokalemia and hypomagnesemia. *Trans. Assoc. Am. Physicians*., **79**, 221–235.

Grantham, J.J. & Orloff, J. (1968) Effect of prostaglandin E_1 on the permeability response of the isolated collecting tubule to vasopressin, adenosine 3′,5′-monophosphate, and theophylline. *J. Clin. Invest*., **47**, 1154–1161.

Guo, D.F., Uno, S.M. & Inagami, T. (1995) Steroid hormones upregulate ratangiotensin II type 1A receptor gene: role of glucocorticoid responsive elements in rat angiotensin II type 1A promoter. *J. Steroid. Biochem. Mol. Biol*., **53**, 69–73.

Hebert, S.C. (1999) Molecular mechanisms. *Semi. Nephrol*., **19**, 504–523.

Higaki, J., Katuya, T., Morosita, R. & Ogihara, T. (2001) Hypertension and genes. Symposium on the etiology of hypertension: summarizing studies in 20th century. *Intern. Med*., **40**, 144–147.

Hirata, M., Hayashi, Y., Ushikubi, F., Yokota, Y., Kageyama, R., Nakanishi, S. & Narumiya, S. (1991) Cloning and expression of cDNA for a human thromboxane A_2 receptor. *Nature*, **349**, 617–620.

Hsueh, W.A. & Law, R.E. (2001) PPAR-γ and atherosclerosis: effects on cell growth and movement. *Arterioscler. Thromb. Vasc. Biol*., **21**, 1891–1895.

Ishigami, T., Umemura, S., Tamura, K., Hibi, K., Nyui, N., Kihara, M., Yabana, M., Watanabe, Y., Sumida, Y., Nagahara, T., Ochiai, H. & Ishii, M. (1997) Essenntial hypertension and 5′upstream core prompter region of human angiotensinogene gene. *Hypertension*, **30**, 1325–1330.

Ikeda, Y., Takeuchi, K., Taniyama, Y., Sato, K., Takahashi, N., Sugawara, A. & Ito, S. (1999) Transcriptional suppression of rat angiotensin AT_1a receptor gene expression by interferon-γ in vascular smooth muscle cells. *Biochem. Biophys. Res. Commun*., **262**, 494–498.

Ikeda, Y., Sugawara, A., Taniyama, Y., Uruno, A., Igarashi, K., Arima, A., Ito, S. & Takeuchi, K. (2000) Suppression of rat thromboxane synthase gene transcription by peroxisome proliferator-activated receptor γ in macrophages via an interaction with NRF2. *J. Biol. Chem*., **275**, 33142–33150.

Ikeda, Y., Yamamoto, J., Okamura, M., Fujino, T., Takahashi, S., Takeuchi, K., Osborne, T.F., Yamamoto, T.T., Ito, S. & Sakai, J. (2001) Transcriptional regulation of the murine acetyl-CoA synthetase 1 gene through multiple clustered binding sites for sterol regulatory element-binding proteins and a single neighboring site for Sp1. *J. Biol. Chem*., **276**, 34259–34269.

Lifton, R.P., Aharavi, A.G. & Geller, D.S. (2001) Molecular mechanisms of human hypertension. *Cell*, **104**, 545–556.

Miura, K., Yamakita, N., Yasuda, K., Murase, H., Takeda, N., Takeuchi, K., Yasujima, M., Murakami, O., Abe, K., Yoshinaga, K. & Sasano, N. (1986) Clinical features of dexamethasone-suppressible hyperaldoster-

onism. *Serino Symposia Review*, **10**, 86–104.

Murphy, T.J., Takeuchi K. & Alexander, R.W. (1992) Molecular cloning of AT_1 angiotensin receptors. *Am. J. Hypertens*., **5**, 236S–242S.

Narumiya, S., Sugimoto, Y. & Ushikubi, F. (1999) Prostanoid receptors: structures, properties, and functions. *Physiol. Rev*., **79**, 1193–11226.

Nickenig, G., Roling, J., Strehlow, K., Schnabel, P. & Bohm, M. (1998) Insulin induces upregulation of vascular AT_1 receptor gene expression by posttranscriptional mechanisms. *Circulation*, **98**, 2453–2460.

Ogihara, T., Rakugi, H., Ikegami, H. & Masuo, K. (1995) Enhancement of insulin sensitivity by troglitazone lowers blood pressure in diabetic hypertensives. *Am. J. Hypertens*., **8**, 316–320.

Ohashi, K., Ruan, K.H., Kulmacz, R.J., Wu, K.K. & Wang, L.H. (1992) Primary structure of human thromboxane synthase determined from the cDNA sequence. *J. Biol. Chem*., **267**, 789–793.

Overdier, D.G., Ye, H., Peterson, R.S., Clevidence, D.E. & Costa, R.H. (1997) The winged helix transcriptional activator HFH-3 is expressed in the distal tubules of embryonic and adult mouse kidney. *J. Biol. Chem*., **272**, 13725–13730.

Simon, D.B., Nelson-Williams, C., Bia, M.J., Ellison, D., Karet, F.E., Molina, A.M., Vaara, I., Iwata, F., Cushner, H.M., Koolen, M., Gainza, F.J., Gitelam, H.J. & Lifton, R.P. (1996) Gitelman's variant of Bartter's syndrome, inherited hypokalaemic alkalosis, is caused by mutations in the thiazide-sensitive Na-Cl cotransporter. *Nat. Genet*., **12**, 24–30.

Sugawara, A., Takeuchi, K., Uruno, A., Ikeda, Y., Arima, S., Sato, K., Taniyama, Y. & Ito, S. (2001a) Transcriptional suppression of type 1 angiotensin II receptor gene expression by peroxisome proliferator-activated receptor-γ in vascular smooth muscle cells. *Endocrinology*, **142**, 3125–3134.

Sugawara, A., Takeuchi, K., Uruno, A., Ikeda, Y., Arima, S., Sato, K., Taniyama, Y. & Ito, S. (2001b) Differential effects among thiazolidinediones on the transcription of thromboxane receptor and angiotensin II type 1 receptor genes. *Hypertens. Res*., **24**, 229–233.

Sugawara, A., Uruno, A., Kudo, Y., Ikeda, Y., Sato, K., Taniyama, Y., Ito, S. & Takeuchi, K. (2002) Transcription suppression of thromboxane receptor gene by peroxisome proliferator-activated receptor-γ via an interaction with Sp1 in vascular smooth muscle cells. *J. Biol. Chem*., **277**, 96776–96783.

Takahashi, N., Takeuchi, K., Abe, T., Murakami, K., Yamaguchi, M. & Abe, K. (1995) Immunohistochemical localization of thromboxane receptor and thromboxane synthase in rat testis. *Endocrinology*, **136**, 4143–4146.

Takahashi, N., Takeuchi, K., Sugawara, A. & Abe, K. (1996) Immunolocalization of rat thromboxane receptor in the kidney. *Endocrinology*, **137**, 5170–5173.

Takahashi, N., Takeuchi, K., Sugawara, A., Taniyama, Y., Kato, T., Wilcox, C.S., Abe, K. & Ito, S. (1998) Structure and transcriptional function of the 5′-flanking region of rat thromboxane receptor gene. *Biochem. Biophys. Res. Commun*., **244**, 489–493.

Takeuchi, K., Abe, K., Sato, M., Yasujima, M., Omata, K., Hagino, T., Fang, S. & Yoshinaga, K. (1989) Plasma aldosterone levels in a female case of pseudohyperaldosteronism (Liddle's Syndrome). *Endocrinol. Japon*., **36**, 167–173.

Takeuchi, K., Nakamura, N., Cook, N., Pratt, R.E. & Dzau, J. (1990) Angiotensin II can regulate gene expression by the AP-1 binding sequence via a protein kinase C dependent pathway. *Biochem. Biophys. Res. Commun*., **172**, 1189–1194.

Takeuchi, K., Abe, K., Yasujima, M., Sato, S., Maeyama, K., Watanabe, T., Sato, M., Inaba, H. & Yoshinaga, K. (1992) Phosphoinositide hydrolysis and calcium mobilization induced by angiotensin II and vasopressin in vascular smooth muscle. *Tohoku J. Exp. Med*., **166**, 107–122.

Takeuchi, K., Alexander, R.W., Nakamura, Y., Tsujino, T. & Murphy, T.J. (1993a) Molecular structure and transcriptional function of the rat vascular AT_1a angiotensin receptor gene. *Circ. Res*., **73**, 612–621.

Takeuchi, K., Abe T., Takahashi, N. & Abe, K. (1993b) Molecular cloning and intrarenal localization of the rat prostaglandin E_2 receptor EP_3 subtype. *Biochem. Biophys. Res. Comm*., **194**, 885–891.

Takeuchi, K., Yanai, N., Takahashi, N., Abe, T., Tsutsumi, E. & Abe, K. (1994a) Different cellular mechanisms of vasopressin receptor V_1 and V_2 subtype in vasopressin-induced adenosine 3′,5′-monophosphate formation in

an immortalized reanal tubule cell line, TKC2. *Biochem. Biophys. Res. Comm*., **202**, 680–687.

Takeuchi, K., Takahashi, N., Abe, T., Ito, O., Tsutumi, E., Taniyama, Y. & Abe, K. (1994b) Functional diffeence between two isoforms of rat kidney prostaglandin receptor EP_3 subtype. *Biochem. Biophys. Res. Comm*., **203**, 1897–1903.

Takeuchi, K., Takahashi, N., Kato, T., Abe, T., Tsutsumi, E., Ito, O., Taniyama, Y. & Abe, K. (1996a) Functional analysis and chromosomal gene assignment of rat prostaglandin EP_3 receptor. *Kidney Int*., **49**, S183–S186.

Takeuchi, K., Kure, S., Kato, T., Taniyama, Y., Takahashi, N., Ikeda, Y., Abe, T., Narisawa, K., Muramatsu, Y. & Abe, K. (1996b) Association of a mutation in thiazide-sensitive Na-Cl cotransporter with familial Gitelman's syndrome. *J. Clin. Endocrinol. Metab*., **81**, 4496–4499.

Takeuchi, K., Nakagawara, K., Mori, M., Abe, T., Takahashi, N., Tsustumi, E., Taniyama, Y., Kato, T., Takeuchi, T. & Abe, K. (1996c) Assignment of the gene for rat thromboxane receptor (Tbxa2r) to chromosome 7q11 by fluorescence in situ hybridization. *Cytogenet. Cell Genet*., **73**, 79–80.

Takeuchi, K., Tsutsumi, E., Abe, T., Takahashi, N., Kato, T., Taniyama, Y., Ikeda, Y. & Abe, K. (1997a) Assignment of rat thromboxane synthase gene (Tbxas) to chromosome 4q21–q22 by fluorescence in situ hybridization. *Cytogenet. Cell Genet*., **76**, 47–48.

Takeuchi, K., Kato, T., Taniyama, Y., Tsunoda, K., Takahashi, N., Ikeda, Y., Omata, K., Imai, Y., Saito, T., Ito, S. & Abe, K. (1997b) Three cases of Gitelman's syndrome possibly caused by different mutations in the thiazide-sensitive Na-Cl cotransporter. *Intern. Med*., **36**, 582–585.

Taniyama, Y., Takeuchi, K., Sugawara, A., Ikeda, Y., Sato, K. & Ito, S. (2000a) Loss of function by a mutation in thiazide-sensitive Na-Cl cotransporter found in Gitelman's syndrome. In: *Control and Diseases of Sodium Dependent Transporter Proteins and Ion Channels*, edited. by E.E. Carafoli, M. Lazdunski, M. Mikoshiba, Y. Okada, Y. Suketa and E.M. Wright, Elsevier Science B.V., Amsterdam, The Netherlands, pp. 55–56.

Taniyama, Y., Takeuchi, K., Sugawara, A., Ikeda, Y., Sato, K., Kondo, Y., Kudo, A., Uruno, A. & Ito, S. (2000b) Effect of steroid hormones on gene transcription of thiazide-sensitive Na-Cl cotransporter. In: *Control and Diseases of Sodium Dependent Transporter Proteins and Ion Channels*, edited by E. Carafoli, M. Lazdunski, M. Mikoshiba, Y. Okada, Y. Suketa and E.M. Wright, Elsevier Science B.V., Amsterdam, The Netherlands, pp. 53–54.

Taniyama, Y., Sato, K., Sugawara, A., Uruno, A., Ikeda, Y., Kudo, Y., Ito, S. & Takeuchi, K. (2001) Renal tubule-specific transcription and chromosomal localization of rat thiazide-sensitive Na-Cl cotransporter gene. *J. Biol. Chem*., **276**, 26260–26268.

Tsutsumi, E., Takeuchi, K., Abe, T., Takahashi, N., Kato, T., Taniyama, Y., Ikeda, Y., Ito, S. & Abe, K (1997) Rat kidney thromboxane synthase: cDNA cloning and gene expression regulation in hydronephrotic kidney. *Prostaglandins*, **53**, 423–431.

Ushikubi, F., Nakajima, M., Hirata, M., Okuma, M., Fujiwara, M. & Narumiya, S. (1989) Purification of the thromboxane A_2/prostaglandin H_2 receptor from human blood platelets. *J. Biol. Chem*., **264**, 16496–16501.

Velloso, L.A., Folli, S., Sun, X.J., White, M.F., Saad, M.J. & Kahn, C.R. (1996) Cross-talk between the insulin and angiotensin signaling systems. *Proc. Natl. Acad. Sci. USA*, **93**, 12490–12495.

Welch, W.J., Peng, B., Takeuchi, K. & Wilcox, C.S. (1997) Salt loading enhances rat renal TxA_2/PGH_2 receptor expression and TGF response to U-46,619. *Am. J. Physiol*., **279**, F976–F983.

White, P.C., Mune, T. & Agarwal, A.K. (1997) 11β-Hydroxysteroid dehydrogenase and the syndrome of apparent mineralocorticoid excess. *Endocr. Rev*., **18**, 135–156.