Genetic Modifications to Alter Blood Pressure Level

Subjects: Peripheral Vascular Disease

Contributor: Hiroki Ohara , Toru Nabika

Genetic manipulation is one of the indispensable techniques to examine gene functions both in vitro and in vivo. In particular, cardiovascular phenotypes such as blood pressure cannot be evaluated in vitro system, necessitating the creation of transgenic or gene-targeted knock-out and knock-in experimental animals to understand the pathophysiological roles of specific genes on the disease conditions.

knock-out genome-editing SHR

1. Introduction

Hypertension is the leading preventable risk factor for cerebro-cardiovascular complications, including heart failure and stroke. Effective anti-hypertensive drugs with different pharmacological actions have been developed; nevertheless, it is deemed that there are 1.28 billion hypertensive patients globally and 0.7 billion or more patients are untreated [1]. Given the resulting mortality and disability as well as the high prevalence, hypertension is still a major public health burden in the world.

It is needless to say that gene-targeted knock-out (KO) and knock-in (KI) or transgenic rodent models have greatly contributed to understanding the pathophysiological basis of hypertension and its vascular complications. In particular, mice have been widely used as the best experimental animal since the gene engineering technique to create KO models was established for over 30 years ago. By contrast, it had been technically difficult to create KO rats for a long time because of the difficulty of rat ES cell culture. Recent advances in genome-editing technologies, however, have made it possible to easily create KO rats similar to mice [2][3]. Given that spontaneous cerebro-cardiovascular disease models, such as Dahl salt-sensitive (SS) and stroke-prone spontaneously hypertensive rats (SHRSP), have been commercially available, a genome-editing strategy using the rat disease models has much potential to clarify the novel pathogenesis of hypertension.

2. Mouse Models

Essential hypertension is a highly complex pathological condition that is formed by synergistic influences of multiple lifestyles, social, environmental, and genetic factors. Since blood pressure (BP) is collaboratively controlled by various organs and tissues, there are many studies that have investigated tissue (or cell)-specific roles of genes on BP regulation using conventional and conditional KO or transgenic mice. In contrast to rats, no spontaneous hypertensive mouse models have been established; accordingly, angiotensin II (Ang II)-infused

models have been widely used to investigate the pathogenesis of Ang II-related hypertension. Deoxycorticosterone acetate (DOCA)-salt or high-salt diet (usually containing 4% or 8% NaCl) models have been also used to investigate the pathogenesis of salt-sensitive hypertension.

2.1. Kidney

The kidney plays pivotal roles in arterial BP regulation by controlling blood volume and plasma electrolyte balance. Activities of the renin–angiotensin–aldosterone system (RAAS) and mineral transporters (Na^+/H^+ exchanger; NHE, $Na^+–K^+–Cl^-$ co-transporter; NKCC, $Na^+–Cl^-$ co-transporter; NCC, epithelial sodium channel; ENaC, etc.) distributed along with nephron are important for physiological BP regulation; thus, genes that may regulate those activities have been widely investigated (**Table 1**).

Ang II regulates BP via Ang II type 1 receptor (*Agtr1a*, AT1R). As BP lowering effects were observed in proximal tubules (PT) or collecting duct (CD)-specific KO mice [4][5], blockade of AT1R signaling in renal epithelial cells would be a pharmacological target for hypertension therapy. Of note, AT1R-associated protein (*Agtrap*), which is widely distributed along renal tubules, has been found to suppress AT1R signaling by facilitating internalization of AT1R resulting in decreased cell surface expression of AT1R [6][7], suggesting that activation of endogenous AGTRAP has potential to reduce BP. In fact, it has been reported that the renal-specific overexpression and conventional KO mice show lower and higher BP phenotype compared with the wild-type (WT) control, respectively [8][9][10][11]. In contrast to the results in mice, however, the deletion in Dahl SS rats exacerbated renal damage under a 4% NaCl diet condition with no change in BP [12]. Although AGTRAP may play double-edged roles in reno-cardiovascular functions in a context-specific manner, it is a potential candidate gene located in a genome-wide association studies (GWAS) loci for BP in humans [12].

Although Ang II is the most well-known bioactive peptide hormone in the RAAS, (pro)renin and Ang-(1-7) produced by angiotensin-converting enzyme 2 (ACE2) are also known to regulate BP via its specific receptors. The (Pro)renin receptor (PRR) that specifically recognizes both prorenin and renin was cloned by Nguyen et al. in 2002 [13]. In the kidney, PRR is mainly expressed in renal vasculature, PT and distal tubules (DT), and CD and enhances the catalytic activity of (pro)renin that converts Ang I to Ang II, resulting in an increase in Ang II production [14]. Consistent with the physiological function of PRR, decreases in BP elevation induced by Ang II infusion have been observed in both tubular- and CD-specific KO mice through inhibition of ENaC activation [15][16][17]. Ang-(1-7) generated by mainly ACE2 is a vasoactive peptide that induces a vasodilation response by binding to Mas receptor [18]. Therefore, ACE2-Ang-(1-7)-Mas axis exerts a counteracting effect on Ang II that causes BP elevation. Ni et al. reported that conventional double KO of both ACE2 and Mas receptor in mice caused greater Ang II-induced BP elevation when compared with the WT littermates [19]. In addition, they also showed that the dual deletion of ACE2 and Mas receptor worsened hypertensive nephropathy, suggesting that ACE2-Ang-(1-7)-Mas receptor axis has protective roles in both the development of hypertension and the resulting hypertensive kidney injury.

Tubuloglomerular feedback (TGF) is an important physiological system to regulate long-term BP by sensing blood volume and electrolyte balance at the level of juxtaglomerular apparatus in each nephron [20]. Accumulating

evidence has shown that local activities of renal oxide synthases (NOS), which produce a major chemical vasodilator NO, play an important role in the regulation of the TGF system. NOS families are composed of three isoforms, i.e., neuronal NOS (nNOS, encoded by *Nos1*), inducible NOS (iNOS, *Nos2*), and endothelial NOS (eNOS, *Nos3*). Although all the three isoforms are expressed in the kidney, *Nos1* and *Nos3* are thought to be major isoforms that physiologically participate in the TGF because of low baseline expression of *Nos2*. Interestingly, Lu et al. showed that macula densa-specific deletion of *Nos1* exacerbated a high-salt diet-induced BP elevation under a condition of Ang II infusion accompanied by reduced glomerular filtration rate (GFR) and Na^+ excretion [21]. It was also reported that local NOS1 activity at the macula densa contributed to a sex difference in BP response to Ang II [22]. Moreover, Hyndman et al. and Gao et al. have investigated renal-specific roles of NOS1 and NOS3 on BP regulation using CD-specific and nephron-specific KO mice, respectively [23][24]. They suggested that deletion of the two isoforms caused greater high-salt-induced BP elevation by enhancing ENaC [25] and NCC activities in the tubular cells, respectively.

Pathophysiological roles of NEDD4-2 (encoded by *Nedd4l*) and with-no-lysine kinases 1 and 4 (*Wnk1* and *Wnk4*) in (salt-sensitive) hypertension have been well-investigated in humans as well as in rodent models. NEDD4-2 is an E3 ubiquitin ligase that ubiquitylates ENaC to down-regulate its cell surface expression and activity [26]. Although NEDD4-2 was initially found as a ENaC-specific regulator in the kidney [25], Ronzaud et al. reported that NEED4-2 also regulated NCC activity and its renal tubule-specific deletion caused salt-dependent hypertension [27]. Consequently, NEED4-2 is involved in the pathogenesis of salt-sensitive hypertension through the two-independent pathways that controls renal Na^+ homeostasis. WNK1 and WNK4 are known to be responsible genes of pseudohypoaldosteronism type 2 (PHA2) that is caused by large deletions in intron 1 of WNK1 or gain-of function mutations in WNK4 [28]. Mechanistically, WNKs phosphorylate SPAK/OSR1, thereby activating NCC in the DT and resulting in increased Na^+ reabsorption and salt-sensitive hypertension [28][29]; however, the molecular network may be a little complicated as a paradoxical role of kidney-specific WNK1 lacking a kinase domain on the development of salt-sensitive hypertension was reported [30]. Moreover, Mu et al. suggested a unique pathway involving salt-sensitive hypertension caused by epigenetic down-regulation of WNK4 [31]. In this context, kelch-like protein 3 (KLHL3) and cullin 3 (CUL3), which are the E3 ubiquitin ligase complex to degrade WNK, have also received much attention as target molecules to prevent salt-sensitive hypertension [28][29].

Unlike the local mechanisms in the kidney described above, Pan et al. uniquely identified the liver–kidney and liver–adipocytes axis to control BP via a hepatocytes-producing hormone, fibroblast growth factor 21 (FGF21), which has pleiotropic effects on glucose and lipid metabolism [32]. They found that FGF21 augmented peroxisome proliferator-activated receptor γ (PPARγ)-mediated activation of ACE2 in both the kidney and adipocytes; thereby, an increase in Ang-(1-7) production reduced both BP and vascular injury. Because FGF21 production was stimulated by Ang II, the FGF21–ACE2 axis may counteract Ang II-induced hypertension and the vascular injury. This might be a key mechanism in obesity-related hypertension.

Besides the above, multiple mechanisms have been proposed such as by circadian clock- [33][34], osmotic stress- [35], and genome-wide association study (GWAS)-related genes [36][37] as well.

Table 1. Target molecules in kidney.

Targets	Type of Genetic Modification	Models	Phenotypes	References
AGTRAP (angiotensin II receptor-associated protein, *Agtrap*)	Renal tubule-specific overexpression	Ang II	↓BP, ↓NCC and αENaC activities	Wakui et al. [8]
	Conventional KO	Ang II	↑BP, ↑ENaC activity	Ohsawa et al. [9]
	Conventional KO	5/6 nephrectomy	↑BP, ↑plasma volume, ↑αENaC and TNF-α expression	Kobayashi et al. [10]
	Proximal tubule-specific KO	Ang II	No differences in basal BP, pressor response to Ang II, and cardiac hypertrophy	Kinguchi et al. [11]
PRR ((Pro)renin receptor, *Atp6ap2*)	Tubular-specific KO	Ang II	↓BP, ↓Na^+ retention, ↓αENaC expression	Ramkumar et al. [15]
	Collecting duct-specific KO	Ang II	↓BP, ↓urinary renin and αENaC activities	Peng et al. [16]
	Collecting duct-specific KO	Ang II	↓BP (basal and Ang II), ↓α/γENaC activation, ↓urinary Ang II and renin levels	Prieto et al. [17]
ACE2 (angiotensin-converting enzyme-2, *Ace2*), Mas receptor (*Mas1*)	Conventional double KO	Ang II	↑BP, ↑ renal injury, ↑serum Cr, ↓Cr clearance	Ni et al. [19]
NOS1 (NO synthase 1, *Nos1*)	Macula densa-specific KO	Ang II + high-salt diet	↑BP, ↑ tubuloglomerular feedback response, ↓GFR, urine flow, and N^+ excretion	Lu et al. [21]
	Macula densa-specific KO	Ang II	Diminished sex difference in Ang II-induced BP, tubuloglomerular feedback response, and natriuretic response	Zhang et al. [22]
	Collecting duct-specific KO	High-salt diet	↑BP, ↓urine output, ↓Na^+, Cl^-, and NOx	Hyndman et al. [23]

Targets	Type of Genetic Modification	Models	Phenotypes	References
			excretion	
NOS3 (NO synthase 3, *Nos3*)	Doxycycline-inducible nephron-specific KO	High-salt diet	↑BP, ↑Na^+ retention, ↑NCC activation	Gao et al. [24]
NEDD4-2 (*Nedd4l*)	Tetracycline-inducible tubule-specific KO	High-salt diet	↑BP, ↑β/γENaC and ROMK expression, ↑NCC activation, hypercalciuria	Ronzaud et al. [27]
WNK1 (with-no-lysine kinase 1, *Wnk1*)	Kidney-specific overexpression of the kidney-specific isoform	No treatment	↓BP, ↑plasma Ang II and aldosterone, ↓NCC and NKCC2 activation	Liu et al. [30]
	Kidney-specific KO (targeted deletion of the first exon of the kidney-specific isoform)	High-salt diet	↑BP, ↑Na^+ retention, ↑NCC and NKCC2 activation	Liu et al. [30]
FGF21 (fibroblast growth factor 21, *Fgf21*)	Conventional KO	Ang II	↑BP, ↑vascular hypertrophy and fibrosis, ↓vascular relaxation, ↓plasma/adipose ACE2 and Ang-(1-7), ↑plasma/adipose Ang II	Pan et al. [32]
BMAL1 (brain and muscle ARNT-like 1, *Arntl*)	Kidney-specific KO	No treatment (or K^+-restricted diet)	↑BP, ↓Na^+ retention under K^+-restricted diet	Crislip et al. [33]
Per1 (period 1, *Per1*)	Distal nephron-specific KO	DOCP-salt	↑BP, ↑Na^+ retention, ↑plasma aldosterone, ↑medullary endothelin-1	Douma et al. [34]
NFAT5 (nuclear factor of activated T-cells 5, *Nfat5*)	Doxycycline-inducible tubular cell-specific KO	High-salt diet	↑BP, hypernatremia, polyuria, ↓Na^+ excretion, ↑ENaC expression	Hiramatsu et al. [35]
HSD11β2 (11β-hydroxysteroid dehydrogenase, *Hsd11b2*)	Kidney-specific KO	No treatment	↑BP, ↑αENaC and NCC activation	Ueda et al. [36]
NPR-C (natriuretic peptide receptor-C, *Npr3*)	Conventional KO	Ang II	↓BP, ↑diuretic and natriuretic response,	Shao et al. [37]

Rats are the generally used experimental animal the same as mice and have some advantages compared with mice such as large body and tissue size and physiological properties similar to those in humans. Despite the

Targets	Type of Genetic Modification	Models	Phenotypes	References
			↓NCC activation via WNK4/SPAK	
	Tubule-specific KO	Ang II	↓BP, ↓NCC activation via WNK4/SPAK [38]	Shao et al. [37]

erimental i.e., zinc terspaced ate KO/KI g body of literature has emerged in the last decade reporting phenotypes of KO/KI rats including genetic hypertensive models as below [39][40].

3.1. KO Models of SHR and SHRSP

SHR (spontaneously hypertensive rat) is a representative genetically hypertensive model that was established by selective breeding of rats with relatively high blood pressure in an outbred colony of Wistar rats that had been maintained in Kyoto University. SHRSP (stroke-prone SHR) is a substrain of SHR that genetically develops more severe hypertension and stroke. Despite the fact that both strains have been widely used for clarifying the responsible genes and the underlying mechanisms of hypertension and its complications [41][42][43], the literature evaluating cardiovascular phenotypes by using KO/KI models are still scant.

SHR is a useful model for hypertensive cardiac hypertrophy [44]. A quantitative trait locus (QTL) related to the left ventricular hypertrophy was previously mapped on chromosome (Chr) 8 by phenotyping of congenic strains between SHR and normotensive Brown Norway (BN) rats [45]. Liška et al. identified promyelocytic leukemia zinc finger (*Plzf*) as a candidate gene on the cardiac QTL and showed that the deletion in SHR did not alter the BP but ameliorated cardiac hypertrophy and fibrosis [46].

Complement 3 (C3) that is overexpressed in aortic smooth muscle cells of SHR has been proposed as a candidate gene responsible for the development of hypertension in this model [47]. Mechanistically, C3-C3a receptor signaling accelerates a change in the characteristic of VSMC and glomerular mesangial cells from contractile to synthetic phenotype via activation of Krüppel-like factor 5 (KLF5) that is a transcription factor to induce the synthetic phenotype of mesenchymal cells [48][49]. Negishi et al. revealed that the C3 deficiency mitigated a salt-sensitive BP elevation and renal injury with decreased renal Ang II level and urinary catecholamine excretion [50].

Rubattu et al. previously identified a QTL on Chr 1 responsible for the susceptibility to salt-induced stroke by a linkage analysis F2 cross between SHR and SHRSP [51]. They identified NADH dehydrogenase (ubiquinone) 1 subunit C2 (*Ndufc2*), encoding a component of the electron transport chain, as a plausible candidate gene in the stroke QTL, then proved that the heterozygous deletion in SHR by ZFN strongly exacerbated the stroke susceptibility with increased oxidative stress and inflammation both in vitro and in vivo [52].

Besides the above, one group recently created peroxiredoxin 2 (*Prdx2*) KO SHR to investigate whether the deletion of an antioxidant gene exacerbates cerebro-cardiovascular phenotypes of SHR [53]. Consequently, *Prdx2* KO SHR had greater basal BP compared with WT SHR. Furthermore, the lifespan of *Prdx2* KO SHR under a salt loading condition was shorter than that of WT SHR despite no difference in BP after salt loading between the KO and the

WT. No apparent inter-strain differences were found in histopathological evaluation for brain, heart, and kidney lesions, and therefore, the reason for the short life span of *Prdx2* KO SHR under the salt loading condition remains fully unknown.

Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is an endothelial scavenger receptor that is closely involved in the pathogenesis of atherosclerosis [54]. Recently, Liang et al. reported that LOX-1 deficiency had a protective role in spontaneous brain damage in SHRSP with no significant change of BP [55].

One group previously found a QTL on Chr1 that affected exaggerated sympathetic responses to the stress of SHRSP by genetic analysis of congenic lines between SHRSP and normotensive Wistar-Kyoto rat (WKY) [56]. Among the genes in the QTL region, stromal interaction molecule 1 (*Stim1*) with a nonsense mutation in SHRSP was identified as a promising candidate [57]. As STIM1 plays a key role in Ca^{2+} homeostasis in the body, it can be expected that the *Stim1* mutation was a genetic determinant responsible for cerebro-cardiovascular traits; however, no significant differences were observed in the sympathetic stress responses as well as age-dependent changes in BP between *Stim1* KI SHRSP and SHRSP, i.e., with WT and mutant allele for *Stim1*, respectively [58]. Phenotyping of the *Stim1* KI SHRSP is currently in progress, and the results will be described elsewhere.

3.2. KO Models of Dahl SS

Dahl salt-sensitive (SS) rats originate from a closed colony of Sprague-Dawley (SD) rats and are widely used as a salt-sensitive hypertension model that develop severe hypertension (>200 mmHg) and the complications such as hypertensive kidney injury and heart failure when fed high-salt diets [59]. SS/Jr and DSS/N strains have been separately established by Rapp and Iwai, respectively. Compared with SHR and SHRSP, multiple KO/KI models with SS/Jr genetic backgrounds have been actively created.

In 2011, Moreno et al. first reported the phenotype of renin KO SS/Jr, in which a severe decrease in basal BP and abnormal kidney morphologies were observed [60]. Thereafter, a growing literature has shown pathophysiological implications of multiple genes on cardiorenal disease traits in SS/Jr [61][62][63][64][65][66][67][68][69][70][71][72][73]. Among them, pleckstrin homology domain containing family A member 7 (*Plekha7*) is a plausible candidate gene for essential hypertension identified by GWAS. A risk variation on *Plekha7*, encoding an adherence junction protein [74], for elevated systolic BP has been found in multiple human populations [75][76][77][78][79]. In this context, Endres et al. created SS/Jr lacking the functional domain of *Plekha7* by ZFN and revealed that the *Plekha7* functional KO SS/Jr had significantly lower BP and renal and cardiac damage under the 8% high-salt diet condition [63]. Their study is a meaningful study that verified a direct effect of the GWAS gene on the hypertensive phenotype in a genetic rat model with salt sensitivity.

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