Disease Models in Rats

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Rats (*Rattus norvegicus*) and mice (*Mus musculus*) belong to the same rodent family and have been the most widely used models in biomedical research for many years. However, there are several differences between these two animals. For example, the rat is larger (roughly about eight- to ten-fold) in size than the mouse, which provides a number of practical advantages, as exemplified by easier and more rapid microsurgery, multiple sampling of larger blood and tissue volumes, and precise injection of substances into blood vessels or the brain (reviewed by Kjell and Olson). Additionally, mice and rats differ in their physiology, behavior, and neurology.

1. Introduction

Rats (*Rattus norvegicus*) and mice (*Mus musculus*) belong to the same rodent family and have been the most widely used models in biomedical research for many years. However, there are several differences between these two animals. For example, the rat is larger (roughly about eight- to ten-fold) in size than the mouse, which provides a number of practical advantages, as exemplified by easier and more rapid microsurgery, multiple sampling of larger blood and tissue volumes, and precise injection of substances into blood vessels or the brain (reviewed by Kjell and Olson [1]). Additionally, mice and rats differ in their physiology, behavior, and neurology. Therefore, rats are considered as useful animals suitable for toxicological, neurobehavioral, and cardiovascular studies compared with other currently available experimental animals (reviewed by Jacob [2]).

There are some examples for the production of genetically modified (GM) rats reported approximately 20 years ago. These GM rats were transgenic (Tg) rats as a "gain of function" model to examine the role of plasma membrane calmodulin-dependent calcium ATPase isoform 4 (*PMCA4*) cDNA under control of the cardiac-specific promoter [3] or sarcoplasmic reticulum Ca^{2+}-ATPase (*SERCA2a*) under control of the ubiquitous promoter [4]. Unfortunately, the production of GM rats, as exemplified by knockout (KO) or knock-in (KI) rats, has not been possible until about 10 years ago. The main reason for this is the inability to establish and maintain rat embryonic stem (ES) cells, which is much more complicated when compared with establishing and maintaining mouse ES cells. For example, in mice, ES cells were already available in the late 1980s [5][6], whereas rat ES cell production began in the late 2000s [7][8]. Since the establishment of rat ES cells, ES cell-mediated gene targeting was performed in rats for the production of KO [9][10][11][12] and KI rats [13][14][15]. For cases in which ES cells are still absent, several alternative approaches, such as transposon-mediated mutagenesis and chemical mutagenesis using N-ethyl-N-nitrosourea, have been developed to assess gene function (reviewed by Zan et al. [16]).

During the period of 2009 to 2013, genome editing (GE) technologies, such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat-associated protein 9 (CRISPR/Cas9) nucleases systems, all of which can induce a double-stranded break (DSB) at a specific site in the genome, appeared; since then, the production of GM rats was accelerated. Using these GE systems, many GM rats have been produced using the direct microinjection (MI) of GE components (including engineered endonucleases) into the cytoplasm or nucleus of a zygote (fertilized one-cell embryo), in vitro electroporation (EP) of isolated zygotes in the presence of GE components, and in vivo EP after the instillation of GE components into the oviductal lumen of a pregnant female (corresponding to zygote to the 2-cell stage), which is termed "genome-editing via oviductal nucleic acids delivery (GONAD) (or *improved* GONAD (*i*-GONAD))". Furthermore, it has become possible to engineer the genome of cultured spermatogonial stem cells (SSCs), precursor cells for spermatogenesis. The transplantation of these GM SSCs into the recipient male rat testes and subsequent mating of these transplanted males with wild-type (WT) females led to the production of GM rats.

2. Disease Models in Rats

For a decade, the advent of genetic engineering tools such as ZFNs, TALENs, and CRISPR/Cas9 has led to a revolution in obtaining specific and targeted genetic mutations in rats for the study of human genetic diseases. Several important genetic diseases have been modeled in rats. A brief description of the most useful models is provided below.

2.1. Models for Cardiovascular Diseases

Monoallelic mutations in the gene encoding bone morphogenetic protein receptor 2 (*Bmpr2*) are the main genetic risk factors for heritable pulmonary arterial hypertension (PAH) with incomplete penetrance. Several *Bmpr2* Tg mice have been reported to develop mild spontaneous PAH. Ranchoux et al. [17] generated *Bmpr2* KO rats using the ZFN technology. The resulting KO rats with a heterozygous 140 bp deletion in the first exon of *Bmpr2* displayed intense pulmonary vascular remodeling. The same group [18] also generated rat lines with mutations (deletion of 71 bp in exon 1 of *Bmpr2*) and showed that the heterozygous rats developed age-dependent spontaneous PAH with a low penetrance (16–27%), similar to that in humans. They concluded that this new genetic rat model represents a promising tool to study PAH pathogenesis. Concerning the pathogenesis of PAH, mutations in the potassium channel subfamily K member 3 (*KCNK3*) gene, which encodes an outward rectifier K+ channel, have been identified in PAH patients. Lambert et al. [19] generated *Kcnk3* KO rats through the CRISPR/Cas9 technology. The resulting rats developed age-dependent PAH associated with low serum-albumin concentrations. Lambert et al. [19] concluded that KCNK3 loss of function is a key event in PAH pathogenesis.

2.2. Models for Neurological Diseases

Animal models are required to understand the pathogenesis of autism spectrum disorder (ASD). Despite the apparent advantages of mice for neural studies, rats have not been widely used for ASD studies, probably owing to the lack of convenient genome manipulation tools. Hamilton et al. [20] generated two rat models for ASD, one syndromic and one non-syndromic model, through the ZFN system, by destroying a gene (*Fmr1*) coding the Fragile X mental retardation protein (FMRP), the protein responsible for the pathogenesis of Fragile X syndrome (FXS), or a gene (*Ngln3*) coding for Neuroligin3 (NLGN3), a member of the neuroligin synaptic cell-adhesion protein family, respectively. Both FMRP and NLGN3 have been implicated in the pathogenesis of human ASD. Both KO rat lines exhibited abnormalities in ASD-relevant phenotypes including juvenile play, perseverative behaviors, and sensorimotor gating, suggesting the utility of these rats as genetic models for investigating ASD-relevant genes. Later, Tian et al. [21] produced *Fmr1* KO rats through the CRISPR/Cas9 technology targeted to exon 4 of *Fmr1*. Consistent with the previous reports, deletion of the *Fmr1* gene in rats specifically impairs long-term synaptic plasticity and hippocampus-dependent learning in a manner resembling the key symptoms of FXS.

DEP-domain containing 5 gene (*Depdc5*), encoding a repressor of the mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway, has recently emerged as a major gene mutated in familial focal epilepsies and focal cortical dysplasia. Marsan et al. [22] produced a *Depdc5* KO rat using the TALEN technology. Homozygous KO embryos died from Day 14.5 of pregnancy. Heterozygous KO rats developed normally and exhibited no spontaneous electroclinical seizures; however, they showed altered cortical neuron excitability and firing patterns. This rat model is considered a relevant model to study pathogenic mechanisms underlying those disorders.

One subtype of ASD is associated with mutations in the methyl-CpG-binding protein 2 (*Mecp2*) gene, causing an X-linked neurodevelopmental disorder called Rett syndrome (RS). Patients with RS have cognitive defects and circadian clock dysfunction, as exemplified by abnormal sleep patterns. According to Zhai et al. [23], there is an urgent need for new animal models for RS because the existing *Mecp2* KO mouse models fail to fully mimic the pathogenesis and symptoms of patients with RS. *Mecp2* KO rats were successfully produced by the two groups using ZFN [24] and CRISPR/Cas9 [23] technologies. The resulting KO rats exhibited significant abnormalities in growth (body weight loss) as well as behavioral function (anxiety tendency and cognitive deficits) [23][24]. Because these phenotypes well recapitulate the major symptoms of RS patients, these *Mecp2* KO rats will provide an alternative tool for future studies of MeCP2 functions.

Mutations in fused in sarcoma (*Fus*), a nuclear DNA/RNA-binding protein, cause familial ALS and occasionally frontotemporal dementia. Zhang et al. [25] produced KI rats expressing a *Fus* point mutation (R521C) as a model for ALS using the CRISPR/Cas9 technology. The mutant animals developed adult-onset learning and memory behavioral deficits, with reduced spine density in the hippocampal neurons. Remarkably, the sleep–wake cycle and circadian abnormalities preceded the onset of cognitive deficits. These results suggest a new role of *Fus* in sleep and circadian regulation and demonstrate that functional changes in FUS could cause sleep–wake and circadian disturbances as early symptoms.

Emmert et al. [26] produced a novel rat model of X-linked hydrocephalus (XLH) by CRISPR-mediated mutation in the L1 cell-adhesion molecule (*L1cam*) gene on the X chromosome. Hemizygous male mutants developed hydrocephalus and delayed development. The mutant rats did not show reactive gliosis, but exhibited hypomyelination and increased extracellular fluid in the corpus callosum.

GABAergic dysfunctions have been implicated in the pathogenesis of schizophrenia, especially the associated cognitive impairments. The level of the GABA synthetic enzyme glutamate decarboxylase 67 kDa isoform (GAD67) encoded by the *GAD1* gene is downregulated in the brains of schizophrenia patients. Furthermore, a schizophrenia patient harboring a homozygous mutation of *GAD1* has recently been discovered. *Gad1* KO mice exhibited perinatal lethality [27], which precluded characterization at adult stages. Fujihara et al. [28] generated *Gad1* KO rats using the CRISPR/Cas9 technology. Surprisingly, 33% *Gad1* KO rats survived to adulthood, which made further characterization possible. The *Gad1* KO rats exhibited impairments in both spatial reference and working memory without affecting adult neurogenesis in the hippocampus. In addition, *Gad1* KO rats showed a wide range of behavioral alterations, such as enhanced sensitivity to an NMDA receptor antagonist, hypoactivity in a novel environment, and decreased preference for social novelty. These results suggest that *Gad1* KO rats could be a novel model for studying cognitive deficits. Furthermore, Fujihara et al. [28] claimed the necessity to check species differences in the mode of phenotype manifestation when animal models of human diseases are considered.

Angelman syndrome (AS) is a rare genetic disorder characterized by severe intellectual disability, seizures, lack of speech, and ataxia. The gene responsible for AS is the ubiquitin protein ligase E3A (*Ube3a*) gene, which encodes for ubiquitin ligase E6-associated protein (E6AP). Dodge et al. [29] generated *Ube3a* KO rats using the CRISPR/Cas9 system. The resulting KO rats phenotypically mirrored human AS with deficits in motor coordination as well as learning and memory. This model can, thus, offer a new avenue for the study of AS.

Koster et al. [30] produced a pigmented KO rat model for lecithin retinol acyltransferase (LRAT) using the CRISPR/Cas9 system. The introduced mutation (c.12delA) is based on a patient group harboring a homozygous frameshift mutation in the *Lrat* gene (c.12delC), causing a dysfunctional visual (retinoid) cycle. The resulting KO rats exhibited progressively reduced electroretinography potentials from two weeks of age onwards and overall retinal thinning. Vision-based behavioral assays confirmed the reduced vision. These KO rats are a novel animal model for retinal dystrophy, especially for early-onset retinal dystrophies.

2.3. Models for Muscular Diseases

Duchenne muscular dystrophy (DMD) is an X-linked lethal muscle disorder caused by mutations in the Duchenne muscular dystrophy (*Dmd*) gene encoding dystrophin. DMD model animals, such as *Mdx* (X-linked muscular dystrophy) mice and canine X-linked muscular dystrophy dogs, have been widely utilized in the development of a treatment for DMD. However, according to Larcher et al. [31], large animal models such as dogs are expensive and difficult to handle. In contrast, *Mdx* mice only partially mimic the human disease, with limited chronic muscular lesions and muscle weakness. Their small size also imposes limitations on analyses. In this context, a rat model could represent a useful alternative because rats are bigger than mice and could better reflect the lesions and functional abnormalities observed in DMD patients.

Dmd KO rats were successfully produced by two groups using CRISPR/Cas9 technology targeting two exons of the rat *Dmd* [32] or using TALENs targeting exon 23 [31]. These resulting KO rats exhibited a decline in muscle strength and the emergence of degenerative/regenerative phenotypes in the skeletal muscle, heart, and diaphragm. Furthermore, these phenotypes were transmitted to the next generation [32]. Notably, *Dmd* KO rats, but not mice, present cardiovascular alterations close to those observed in humans, which are the main cause of death of patients [31].

Desminopathy is a clinically heterogeneous muscle disease caused by over 60 different mutations in the desmin (*DES*) gene. The most common mutation with a clinical phenotype in humans is an exchange of arginine to proline at position 350 of desmin leading to p.R350P. Langer et al. [33] first produced a KI rat model for a muscle disease through the CRISPR/Cas9 technology using ssODN carrying the missense mutation *Des* c.1045-1046 (AGG > CCG) in exon 6 of *Des*. While muscle weights did not differ between the mutant rats and WT rats, the levels of many muscle-related proteins such as dystrophin, syntrophin, dysferlin, and annexin A2 increased in the mutant rats, showing the phenotype of desminopathy. This rat model will be a useful tool for furthering understanding of the disease and testing therapeutic approaches to delay disease progression.

2.4. Models for Pulmonary Diseases

Cystic fibrosis (CF) is characterized by airway and digestive pathology with a reduced life expectancy and is one of the most common genetic diseases in western populations. The most common mutation is the missense mutation p.Phe508del (or F508del) in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, which leads to abnormal CFTR function and mucus accumulation. Tuggle et al. [34] first generated *Cftr* KO rats using the ZFN technology. The resulting KO rats lacked CFTR activity and exhibited abnormalities in the ileum and increased intracellular mucus in the proximal nasal septa. Airway surface liquid and periciliary liquid depths were reduced, and the submucosal gland size was abnormal. Similar results have also been observed in another *Cftr* KO rat developed by Dreano et al. [35] using the CRISPR/Cas9 technology. Furthermore, Dreano et al. [35] produced another KI rats (carrying F508del mutation). These rats showed residual CFTR activity and milder phenotype than the *Cftr* KO rats. These findings suggest that these rat models can be a CF animal model that recapitulates various aspects of the human disease. Notably, a humanized CF rat strain expressing the G551D variant was produced through KI of the human *CFTR* cDNA carrying the G551D mutation downstream of the endogenous *Cftr* promoter using the ZFN technology [36].

2.5. Models for Metabolic Diseases

The low-density lipoprotein receptor (*LDLR*) and apolipoprotein E (*APOE*) genes control normal levels of cholesterol and other forms of fat in the blood. A deficiency in *ApoE* is involved in several age-related fatty acid diseases. Wei et al. [37] established *ApoE* KO rats through TALEN-mediated gene targeting. After being fed with a high-cholesterol diet (HCD) for 12 weeks, the *ApoE* KO rats displayed typical dyslipidemia, although there was no obvious atherosclerotic lesion in the en face aortas. Notably, partial ligation caused the formation of plaques consisting of lipids and macrophages in carotid arteries from *ApoE* KO rats. Wei et al. [37] concluded that the *ApoE* KO rats can be a novel model for dyslipidemia. Lee et al. [38] produced *ApoE* KO rats using Cas12a (previously named Cpf1), an RNA-guided endonuclease, as a part of the CRISPR system. The resulting KO rats displayed hyperlipidemia and aortic lesions.

A deficiency in *LDLR* is a cause of familial hypercholesterolemia (FH). Zhao et al. [39] and Lee et al. [38] created *Ldlr* KO rats. These *ApoE* and *Ldlr* KO rats mimic pathological changes observed in hyperlipidemia and atherosclerosis in humans with genetic deficiencies and in normal individuals, suggesting usefulness in the research of atherosclerosis.

Melanocortin-3 and -4 receptors (MC3R and MC4R) regulate energy homeostasis. You et al. [40] generated *Mc3r* and *Mc4r* single- and double-KO (DKO) rats using the CRISPR/Cas9 system. *Mc3r* KO rats displayed hypophagia and decreased body weight, whereas *Mc4r* KO and DKO rats exhibited hyperphagia and increased body weight. All three mutants showed increased white adipose tissue mass and adipocyte size. These mutant rats will be important in defining the complicated signaling pathways of MC3R and MC4R. According to You et al. [40], both *Mc4r* KO and DKO rats are good models for obesity and diabetes research.

Hereditary tyrosinemia type I (HT1) is caused by a deficiency in fumarylacetoacetate hydrolase (FAH) enzyme. *Fah*-deficient mice and pigs are phenotypically analogous to human HT1, but do not recapitulate all chronic features of the human disorder, especially liver fibrosis and cirrhosis. Zhang et al. [41] produced *Fah* KO rats through MI of CRISPR/Cas9 components to obtain HT1 models. The *Fah* KO rats faithfully represented hypertyrosinemia, liver failure, and renal tubular damage. More importantly, they developed remarkable liver fibrosis and cirrhosis, which have not been observed in *Fah* mutant mice or pigs. These data suggest that *Fah* KO rats may be used as an animal model of HT1 with liver cirrhosis.

Pseudoxanthoma elasticum (PXE)—a heritable ectopic mineralization disorder—is caused by mutations in the ATP-binding cassette subtype C number 6 (*ABCC6*) gene primarily expressed in the liver and kidneys. These mutations result in generalized arterial calcification throughout the body in infancy. Li et al. [42] generated *Abcc6* KO rats as models of PXE using the ZFN technology. The plasma inorganic pyrophosphate (PPi) level was reduced (<30%), leading to a lowered PPi/inorganic phosphate plasma ratio. When in situ liver and kidney perfusions were performed, the PPi levels in the perfusates were significantly reduced, but those in the liver of WT rats remained high. Li et al. [42] speculate that hepatic ABCC6 may play a critical role in contributing to plasma PPi levels, identifying the liver as a target of molecular correction to counteract ectopic mineralization in PXE.

Wolfram syndrome (WS) is a rare autosomal-recessive disorder caused by mutations in the Wolfram syndrome 1 (*WFS1*) gene and characterized by juvenile-onset diabetes, optic atrophy, hearing loss, and a number of other complications. According to Plaas et al. [43], no mutant *Wfs1* mice displayed fasting hyperglycemia. In other words, previous mouse models of WS involved only partial diabetes and other disease symptoms. Plaas et al. [43] generated *Wfs1* KO rats, in which exon 5 of the *Wfs1* gene was deleted, resulting in a loss of 27 amino acids from the WFS1 protein. The resulting

KO rats showed progressive glucose intolerance, glycosuria, hyperglycemia, and severe body weight loss by 12 months of age. They also exhibited neuronal abnormality such as axonal degeneration and disorganization of the myelin. The phenotype of these KO rats indicates that they have the core symptoms of WS.

Leptin is a cytokine-like hormone principally produced by white adipose tissues. Defects in leptin production cause severe obesity. Leptin receptor (*Lepr*) encoded by the diabetes (*db*) gene is highly expressed in the choroid plexus. Bao et al. [44] produced *Lepr* KO rats through the CRISPR/Cas9 technology. The resulting KO rats exhibited obesity, hyperphagia, hyperglycemia, glucose intolerance, hyperinsulinemia, and dyslipidemia. In contrast, Chen et al. [45] generated *Lepr* KO rats through the TALEN technology. These rat models could complement the existing models (*db/db* mice and Zucker rats) [46][47] and be useful for biomedical and pharmacological research in obesity and diabetes.

Angiopoietin-like protein 8 (ANGPTL8) is a liver- and adipocyte-derived protein that controls plasma triglyceride levels. Izumi et al. [48] generated *Angptl8* KO rats through the CRISPR/Cas9 technology to clarify the roles of *Angptl8* in glucose and lipid metabolism. The resulting KO rats exhibited decreased body weight and fat content, associated with impaired lipogenesis in adipocytes. Izumi et al. [48] suggest that ANGPTL8 might be an important therapeutic target for obesity and dyslipidemia.

Hereditary aceruloplasminemia (HA) is a genetic disease characterized by iron accumulation in the liver and brain. The mutation of the ceruloplasmin (*Cp* gene is thought to be related to HA pathogenesis. Kenawi et al. [49] generated *Cp* KO rats through the CRISPR/Cas9 system. The *Cp* KO rats exhibited decreased iron concentration, transferrin saturation, plasma ceruloplasmin, and ferroxidase activity, which is considered essential for macrophage iron release. Thus, *Cp* KO rats can mimic the iron hepatosplenic phenotype in HA, which will form the basis to understand and treat the disease.

2.6. Models for Kidney Diseases

The renin (REN)-angiotensin system plays an important role in the control of blood pressure and renal function. Moreno et al. [50] produced *Ren* KO rats through ZFN-mediated GE system targeted to exon 5 of *Ren*. The resulting rats exhibited reduced body weight, lower blood pressure, and abnormal renal morphology (as exemplified by cortical interstitial fibrosis and abnormally shaped glomeruli). These results suggest the role of REN in the regulation of blood pressure and kidney function.

Primary hyperoxaluria type 1 (PH1) is an inherited disease caused by mutations in the mitochondrial localized alanine-glyoxylate aminotransferase (*Agxt*) gene, leading to abnormal metabolism of glyoxylic acid in the liver, subsequent endogenous oxalate overproduction, and deposition of oxalate in multiple organs, mainly the kidney. Patients with PH1 often suffer from recurrent urinary tract stones and finally renal failure. There is no effective treatment other than combined liver–kidney transplantation. Zheng et al. [51] produced *Agxt* KO rats as PH1 models through the CRISPR/Cas9 system. The resulting *Agxt* KO rats excreted more oxalate in the urine than WT animals and exhibited crystalluria with mild fibrosis in the kidney. These data suggest that *Agxt*-deficiency in mitochondria impairs glyoxylic acid metabolism and leads to PH1 in rats. This rat strain would be a valuable tool for developing innovative drugs and therapeutics.

Urate oxidase (uricase) encoded by *UOX* gene is a key enzyme whose disfunction causes hyperuricemia. Because of the low survival rate of *Uox*-deficient mice [52], Yu et al. [53] generated *Uox* KO rats through the CRISPR/Cas9 system targeting the exons 2 to 4 of *Uox*. The resulting *Uox* KO rats, called "Kunming-DY rats", were apparently healthy, with more than a 95% survival up to one year. The male rats' serum uric acid increased at levels significantly higher than those of WT rats. Kunming-DY rats exhibited histological renal changes including mild glomerular/tubular lesions, suggesting an alternative model animal to study hyperuricemia and associated diseases mimicking human conditions.

2.7. Models for Ophthalmology Diseases

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor which plays a role in the development of multiple tissues and is activated by a large number of ligands, including 2,3,7,8-tetrachlorodibenzo-p-dioxin. To examine the roles of the AHR in both normal biological development and response to environmental chemicals, Harrill et al. [54] produced *Ahr* KO rats through the ZFN technology targeting exon 2 of the *Ahr* and compared with an existing *Ahr* KO mouse model. *Ahr* KO rats, but not *Ahr* KO mice, displayed pathological alterations to the urinary tract, as exemplified by bilateral renal dilation (hydronephrosis). In contrast, abnormalities in vascular development were observed in *Ahr* KO mice, but not in rats. These findings suggest the differences in the role of AHR in tissue development, homeostasis, and toxicity between rats and mice.

2.8. Models for Hematological Systems

Hemophilia A is a genetic bleeding disorder resulting from factor VIII (FVIII or F8) deficiency. In preclinical hemophilia research, an animal model that reflects both the phenotype and pathology of the disease is required. Nielsen et al. [55] produced KO rats lacking detectable F8 activity through the ZFN technology targeting exon 16 of the *F8* gene. Episodes of spontaneous bleeding requiring treatments were observed in 70% of *F8* KO rats. Shi et al. [56] produced *F8* KO rats in which nearly the entire rat *F8* gene was inverted, causing translational stop six amino acids after the signal sequence, through the CRISPR/Cas9 technology to examine whether platelet F8 expression can prevent severe spontaneous bleeding in *F8* KO rats. They showed that the severe spontaneous bleeding phenotype in *F8* KO rats was successfully rescued by platelet-specific F8 expression through bone marrow cell transplantation.

Autoimmune regulator (AIRE) deficiency in humans induces a life-threatening generalized autoimmune disease called autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED), and no curative treatments are available. Several models of AIRE-deficient mice have been generated; although they have been useful in understanding the role of AIRE in central tolerance, they do not reproduce the APECED symptoms accurately and, thus, there is still a need for an animal model displaying APECED-like disease. Ossart et al. [57] produced an *Aire* KO rat model using ZFN technology. The resulting KO rats exhibited several of the key symptoms of APECED disease, including alopecia, skin depigmentation, and nail dystrophy, which are much more pronounced than those in *Aire* KO mice and closer to manifestations in humans.

2.9. Others

Estrogens play pivotal roles in the development and function of many organ systems, including the reproductive system. Rumi et al. [58] generated estrogen receptor 1 (*Esr1*) KO rats using the ZFN system targeting exon 3 of *Esr1*. Both male and female *Esr1* KO rats were infertile. *Esr1* KO males had small testes with distended and dysplastic STs, whereas *Esr1* KO females possessed large polycystic ovaries, thread-like uteri, and poorly developed mammary glands. In addition, the uteri of *Esr1* KO rats failed to 17β-estradiol treatment. This rat model provides a new experimental tool for investigating the pathophysiology of estrogen action.

The forkhead box N1 (*FOXN1*) gene is known as a critical factor for the differentiation of thymic and skin epithelial cells. Goto et al. [59] generated *Foxn1* KO rats through the CRISPR/Cas9 technology. The resulting *Foxn1* KO rats exhibited thymus deficiency and incomplete hairless, which was characterized by splicing variants.

Multidrug resistance 1 (MDR1; also known as P-glycoprotein) is a key efflux transporter that plays an important role not only in the transport of endogenous and exogenous substances, but also in tumor MDR, one of the most important impediments to the effective chemotherapy of cancer. In rodents, two isoforms, *Mdr1a* and *Mdr1b*, encode MDR1. Liang et al. [60] produced DKO rats (in which both *Mdr1a* and *Mdr1b* had been disrupted) through the CRISPR/Cas9 system. Pharmacokinetic studies of digoxin, a typical substrate of MDR1, confirmed the deficiency of MDR1 in resulting KO rats. This rat model is a useful tool for studying the function of MDR1 in drug absorption, tumor MDR, and drug-target validation.

References

1. Kjell, J.; Olson, L. Rat models of spinal cord injury: From pathology to potential therapies. Dis. Models Mech. 2016, 9, 1125–1137.

2. Jacob, H.J. The Rat: A model used in biomedical research. In Rat Genomics: Methods and Protocols Methods in Molecular Biology; Anegon, I., Ed.; Humana Press: Totowa, NJ, USA, 2010; pp. 1–11.

3. Hammes, A.; Oberdorf-Maass, S.; Rother, T.; Nething, K.; Gollnick, F.; Linz, K.W.; Meyer, R.; Hu, K.; Han, H.; Gaudron, P.; et al. Overexpression of the sarcolemmal calcium pump in the myocardium of transgenic rats. Circ. Res. 1998, 83, 877–888.

4. Vetter, R.; Rehfeld, U.; Reissfelder, C.; Weifl, W.; Wagner, K.D.; Günther, J.; Hammes, A.; Tschöpe, C.; Dillmann, W.; Paul, M. Transgenic overexpression of the sarcoplasmic reticulum Ca2+ ATPase improves reticular Ca2+ handling in normal and diabetic rat hearts. FASEB J. 2002, 16, 1657–1659.

5. Thomas, K.R.; Capecchi, M.R. Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. Cell 1987, 51, 503–512.

6. Doetschman, T.; Gregg, R.G.; Maeda, N.; Hooper, M.L.; Melton, D.W.; Thompson, S.; Smithies, O. Targetted correction of a mutant HPRT gene in mouse embryonic stem cells. Nature 1987, 330, 576–578.

7. Buehr, M.; Meek, S.; Blair, K.; Yang, J.; Ure, J.; Silva, J.; McLay, R.; Hall, J.; Ying, Q.L.; Smith, A. Capture of authentic embryonic stem cells from rat blastocysts. Cell 2008, 135, 1287–1298.

8. Li, P.; Tong, C.; Mehrian-Shai, R.; Jia, L.; Wu, N.; Yan, Y.; Maxson, R.E.; Schulze, E.N.; Song, H.; Hsieh, C.L.; et al. Germline competent embryonic stem cells derived from rat blastocysts. Cell 2008, 135, 1299–1310.

9. Kawamata, M.; Ochiya, T. Generation of genetically modified rats from embryonic stem cells. Proc. Natl. Acad. Sci. USA 2010, 107, 14223–14228.

10. Meek, S.; Buehr, M.; Sutherland, L.; Thomson, A.; Mullins, J.J.; Smith, A.J.; Burdon, T. Efficient gene targeting by homologous recombination in rat embryonic stem cells. PLoS ONE 2010, 5, e14225.

11. Tong, C.; Li, P.; Wu, N.L.; Yan, Y.; Ying, Q.L. Production of p53 gene knockout rats by homologous recombination in embryonic stem cells. Nature 2010, 467, 211–213.

12. Lan, H.; Li, S.; Guo, Z.; Men, H.; Wu, Y.; Li, N.; Bryda, E.C.; Capecchi, M.R.; Wu, S. Efficient generation of selection-gene-free rat knockout models by homologous recombination in ES cells. FEBS Lett. 2016, 590, 3416–3424.

13. Kobayashi, T.; Yamaguchi, T.; Hamanaka, S.; Kato-Itoh, M.; Yamazaki, Y.; Ibata, M.; Sato, H.; Lee, Y.S.; Usui, J.; Knisely, A.S.; et al. Generation of rat pancreas in mouse by interspecific blastocyst injection of pluripotent stem cells. Cell 2010, 142, 787–799.

14. Yamamoto, S.; Ooshima, Y.; Nakata, M.; Yano, T.; Nishimura, N.; Nishigaki, R.; Satomi, Y.; Matsumoto, H.; Matsumoto, Y.; Takeyama, M. Efficient gene-targeting in rat embryonic stem cells by CRISPR/Cas and generation of human kynurenine aminotransferase II (KAT II) knock-in rat. Transgenic Res. 2015, 24, 991–1001.

15. Chen, Y.; Spitzer, S.; Agathou, S.; Karadottir, R.T.; Smith, A. Gene editing in rat embryonic stem cells to produce in vitro models and in vivo reporters. Stem Cell Rep. 2017, 9, 1262–1274.

16. Zan, Y.; Haag, J.D.; Chen, K.S.; Shepel, L.A.; Wigington, D.; Wang, Y.R.; Hu, R.; Lopez-Guajardo, C.C.; Brose, H.L.; Porter, K.I.; et al. Production of knockout rats using ENU mutagenesis and a yeast-based screening assay. Nat. Biotechnol. 2003, 21, 645–651.

17. Ranchoux, B.; Antigny, F.; Rucker-Martin, C.; Hautefort, A.; Péchoux, C.; Bogaard, H.J.; Dorfmüller, P.; Remy, S.; Lecerf, F.; Planté, S.; et al. Endothelial-to-mesenchymal transition in pulmonary hypertension. Circulation 2015, 131, 1006–1018.

18. Hautefort, A.; Mendes-Ferreira, P.; Sabourin, J.; Manaud, G.; Bertero, T.; Rucker-Martin, C.; Riou, M.; Adão, R.; Manoury, B.; Lambert, M.; et al. Bmpr2 mutant rats develop pulmonary and cardiac characteristics of pulmonary arterial hypertension. Circulation 2019, 139, 932–948.

19. Lambert, M.; Capuano, V.; Boet, A.; Tesson, L.; Bertero, T.; Nakhleh, M.K.; Remy, S.; Anegon, I.; Pechoux, C.; Hautefort, A.; et al. Characterization of Kcnk3-mutated rat, a novel model of pulmonary hypertension. Circ. Res. 2019, 125, 678–695.

20. Hamilton, S.M.; Green, J.R.; Veeraragavan, S.; Yuva, L.; McCoy, A.; Wu, Y.; Warren, J.; Little, L.; Ji, D.; Cui, X.; et al. Fmr1 and Nlgn3 knockout rats: Novel tools for investigating autism spectrum disorders. Behav. Neurosci. 2014, 128, 103–109.

21. Tian, Y.; Yang, C.; Shang, S.; Cai, Y.; Deng, X.; Zhang, J.; Shao, F.; Zhu, D.; Liu, Y.; Chen, G.; et al. Loss of FMRP impaired hippocampal long-term plasticity and spatial learning in rats. Front. Mol. Neurosci. 2017, 10, 269.

22. Marsan, E.; Ishida, S.; Schramm, A.; Weckhuysen, S.; Muraca, G.; Lecas, S.; Liang, N.; Treins, C.; Pende, M.; Roussel, D.; et al. Depdc5 knockout rat: A novel model of mTORopathy. Neurobiol. Dis. 2016, 89, 180–189.

23. Zhai, W.; Hu, H.; Le, L.; Zhuang, F.; Wang, K.; Zhao, Y.; Wang, K.Z.; Liu, X.M.; Sun, D.; Wang, X.Y.; et al. Generation and analysis of the Rett syndrome-associated MeCP2-null rat model. Yi Chuan 2016, 38, 1004–1011.

24. Patterson, K.C.; Hawkins, V.E.; Arps, K.M.; Mulkey, D.K.; Olsen, M.L. MeCP2 deficiency results in robust Rett-like behavioural and motor deficits in male and female rats. Hum. Mol. Genet. 2016, 25, 3303–3320.

25. Zhang, T.; Jiang, X.; Xu, M.; Wang, H.; Sang, X.; Qin, M.; Bao, P.; Wang, R.; Zhang, C.; Lu, H.; et al. Sleep and circadian abnormalities precede cognitive deficits in R521C FUS knockin rats. Neurobiol. Aging 2018, 72, 159–170.

26. Emmert, A.S.; Vuong, S.M.; Shula, C.; Lindquist, D.; Yuan, W.; Hu, Y.C.; Mangano, F.T.; Goto, J. Characterization of a novel rat model of X-linked hydrocephalus by CRISPR-mediated mutation in L1cam. J. Neurosurg. 2019, 132, 945–958.

27. Asada, H.; Kawamura, Y.; Maruyama, K.; Kume, H.; Ding, R.G.; Kanbara, N.; Kuzume, H.; Sanbo, M.; Yagi, T.; Obata, K. Cleft palate and decreased brain gamma-aminobutyric acid in mice lacking the 67-kDa isoform of glutamic acid

decarboxylase. Proc. Natl. Acad. Sci. USA 1997, 94, 6496–6499.

28. Fujihara, K.; Yamada, K.; Ichitani, Y.; Kakizaki, T.; Jiang, W.; Miyata, S.; Suto, T.; Kato, D.; Saito, S.; Watanabe, M.; et al. CRISPR/Cas9-engineered Gad1 elimination in rats leads to complex behavioral changes: Implications for schizophrenia. Transl. Psychiatry 2020, 10, 426.

29. Dodge, A.; Peters, M.M.; Greene, H.E.; Dietrick, C.; Botelho, R.; Chung, D.; Willman, J.; Nenninger, A.W.; Ciarlone, S.; Kamath, S.G.; et al. Generation of a novel rat model of Angelman syndrome with a complete Ube3a gene deletion. Autism Res. 2020, 13, 397–409.

30. Koster, C.; van den Hurk, K.T.; Lewallen, C.F.; Talib, M.; Ten Brink, J.B.; Boon, C.; Bergen, A.A. The Lrat−/− rat: CRISPR/Cas9 construction and phenotyping of a new animal model for retinitis pigmentosa. Int. J. Mol. Sci. 2021, 22, 7234.

31. Larcher, T.; Lafoux, A.; Tesson, L.; Remy, S.; Thepenier, V.; François, V.; Le Guiner, C.; Goubin, H.; Dutilleul, M.; Guigand, L.; et al. Characterization of dystrophin deficient rats: A new model for Duchenne muscular dystrophy. PLoS ONE 2014, 9, e110371.

32. Nakamura, K.; Fujii, W.; Tsuboi, M.; Tanihata, J.; Teramoto, N.; Takeuchi, S.; Naito, K.; Yamanouchi, K.; Nishihara, M. Generation of muscular dystrophy model rats with a CRISPR/Cas system. Sci. Rep. 2014, 4, 5635.

33. Langer, H.T.; Mossakowski, A.A.; Willis, B.J.; Grimsrud, K.N.; Wood, J.A.; Lloyd, K.; Zbinden-Foncea, H.; Baar, K. Generation of desminopathy in rats using CRISPR-Cas9. J. Cachexia Sarcopenia Muscle 2020, 11, 1364–1376.

34. Tuggle, K.L.; Birket, S.E.; Cui, X.; Hong, J.; Warren, J.; Reid, L.; Chambers, A.; Ji, D.; Gamber, K.; Chu, K.K.; et al. Characterization of defects in ion transport and tissue development in cystic fibrosis transmembrane conductance regulator (CFTR)-knockout rats. PLoS ONE 2014, 9, e91253.

35. Dreano, E.; Bacchetta, M.; Simonin, J.; Galmiche, L.; Usal, C.; Slimani, L.; Sadoine, J.; Tesson, L.; Anegon, I.; Concordet, J.P.; et al. Characterization of two rat models of cystic fibrosis—KO and F508del CFTR—Generated by Crispr-Cas9. Anim. Models Exp. Med. 2019, 2, 297–311.

36. Birket, S.E.; Davis, J.M.; Fernandez-Petty, C.M.; Henderson, A.G.; Oden, A.M.; Tang, L.; Wen, H.; Hong, J.; Fu, L.; Chambers, A.; et al. Ivacaftor reverses airway mucus abnormalities in a rat model harboring a humanized G551D-CFTR. Am. J. Respir. Crit. Care Med. 2020, 202, 1271–1282.

37. Wei, S.; Zhang, Y.; Su, L.; He, K.; Wang, Q.; Zhang, Y.; Yang, D.; Yang, Y.; Ma, S. Apolipoprotein E-deficient rats develop atherosclerotic plaques in partially ligated carotid arteries. Atherosclerosis 2015, 243, 589–592.

38. Lee, J.G.; Ha, C.H.; Yoon, B.; Cheong, S.A.; Kim, G.; Lee, D.J.; Woo, D.C.; Kim, Y.H.; Nam, S.Y.; Lee, S.W.; et al. Knockout rat models mimicking human atherosclerosis created by Cpf1-mediated gene targeting. Sci. Rep. 2019, 9, 2628.

39. Zhao, Y.; Yang, Y.; Xing, R.; Cui, X.; Xiao, Y.; Xie, L.; You, P.; Wang, T.; Zeng, L.; Peng, W.; et al. Hyperlipidemia induces typical atherosclerosis development in Ldlr and Apoe deficient rats. Atherosclerosis 2018, 271, 26–35.

40. You, P.; Hu, H.; Chen, Y.; Zhao, Y.; Yang, Y.; Wang, T.; Xing, R.; Shao, Y.; Zhang, W.; Li, D.; et al. Effects of melanocortin 3 and 4 receptor deficiency on energy homeostasis in rats. Sci. Rep. 2016, 6, 34938.

41. Zhang, L.; Shao, Y.; Li, L.; Tian, F.; Cen, J.; Chen, X.; Hu, D.; Zhou, Y.; Xie, W.; Zheng, Y.; et al. Efficient liver repopulation of transplanted hepatocyte prevents cirrhosis in a rat model of hereditary tyrosinemia type I. Sci. Rep. 2016, 6, 31460.

42. Li, Q.; Kingman, J.; van de Wetering, K.; Tannouri, S.; Sundberg, J.P.; Uitto, J. Abcc6 knockout rat model highlights the role of liver in PPi homeostasis in Pseudoxanthoma elasticum. J. Investig. Dermatol. 2017, 137, 1025–1032.

43. Plaas, M.; Seppa, K.; Reimets, R.; Jagomäe, T.; Toots, M.; Koppel, T.; Vallisoo, T.; Nigul, M.; Heinla, I.; Meier, R.; et al. Wfs1-deficient rats develop primary symptoms of Wolfram syndrome: Insulin-dependent diabetes, optic nerve atrophy and medullary degeneration. Sci. Rep. 2017, 7, 10220.

44. Bao, D.; Ma, Y.; Zhang, X.; Guan, F.; Chen, W.; Gao, K.; Qin, C.; Zhang, L. Preliminary characterization of a leptin receptor knockout rat created by CRISPR/Cas9 system. Sci. Rep. 2015, 5, 15942.

45. Chen, Y.; Lu, W.; Gao, N.; Long, Y.; Shao, Y.; Liu, M.; Chen, H.; Ye, S.; Ma, X.; Liu, M.; et al. Generation of obese rat model by transcription activator-like effector nucleases targeting the leptin receptor gene. Sci. China Life Sci. 2017, 60, 152–157.

46. Chua, S.C., Jr.; Chung, W.K.; Wu-Peng, X.S.; Zhang, Y.; Liu, S.M.; Tartaglia, L.; Leibel, R.L. Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. Science 1996, 271, 994–996.

47. Argilés, J.M. The obese Zucker rat: A choice for fat metabolism 1968-1988: Twenty years of research on the insights of the Zucker mutation. Prog. Lipid Res. 1989, 28, 53–66.

48. Izumi, R.; Kusakabe, T.; Noguchi, M.; Iwakura, H.; Tanaka, T.; Miyazawa, T.; Aotani, D.; Hosoda, K.; Kangawa, K.; Nakao, K. CRISPR/Cas9-mediated Angptl8 knockout suppresses plasma triglyceride concentrations and adiposity in rats. J. Lipid Res. 2018, 59, 1575–1585.

49. Kenawi, M.; Rouger, E.; Island, M.L.; Leroyer, P.; Robin, F.; Rémy, S.; Tesson, L.; Anegon, I.; Nay, K.; Derbré, F.; et al. Ceruloplasmin deficiency does not induce macrophagic iron overload: Lessons from a new rat model of hereditary aceruloplasminemia. FASEB J. 2019, 33, 13492–13502.

50. Moreno, C.; Hoffman, M.; Stodola, T.J.; Didier, D.N.; Lazar, J.; Geurts, A.M.; North, P.E.; Jacob, H.J.; Greene, A.S. Creation and characterization of a renin knockout rat. Hypertension 2011, 57, 614–619.

51. Zheng, R.; Fang, X.; He, L.; Shao, Y.; Guo, N.; Wang, L.; Liu, M.; Li, D.; Geng, H. Generation of a primary hyperoxaluria type 1 disease model via CRISPR/Cas9 system in rats. Curr. Mol. Med. 2018, 18, 436–447.

52. Wu, X.; Wakamiya, M.; Vaishnav, S.; Geske, R.; Montgomery, C., Jr.; Jones, P.; Bradley, A.; Caskey, C.T. Hyperuricemia and urate nephropathy in urate oxidase-deficient mice. Proc. Natl. Acad. Sci. USA 1994, 91, 742–746.

53. Yu, Y.; Zhang, N.; Dong, X.; Fan, N.; Wang, L.; Xu, Y.; Chen, H.; Duan, W. Uricase-deficient rat is generated with CRISPR/Cas9 technique. PeerJ 2020, 8, e8971.

54. Harrill, J.A.; Hukkanen, R.R.; Lawson, M.; Martin, G.; Gilger, B.; Soldatow, V.; Lecluyse, E.L.; Budinsky, R.A.; Rowlands, J.C.; Thomas, R.S. Knockout of the aryl hydrocarbon receptor results in distinct hepatic and renal phenotypes in rats and mice. Toxicol. Appl. Pharmacol. 2013, 272, 503–518.

55. Nielsen, L.N.; Wiinberg, B.; Häger, M.; Holmberg, H.L.; Hansen, J.J.; Roepstorff, K.; Tranholm, M. A novel F8−/− rat as a translational model of human hemophilia A. J. Thromb. Haemost. 2014, 12, 1274–1282.

56. Shi, Q.; Mattson, J.G.; Fahs, S.A.; Geurts, A.M.; Weiler, H.; Montgomery, R.R. The severe spontaneous bleeding phenotype in a novel hemophilia A rat model is rescued by platelet FVIII expression. Blood Adv. 2020, 4, 55–65.

57. Ossart, J.; Moreau, A.; Autrusseau, E.; Ménoret, S.; Martin, J.C.; Besnard, M.; Ouisse, L.H.; Tesson, L.; Flippe, L.; Kisand, K.; et al. Breakdown of immune tolerance in AIRE-deficient rats induces a severe autoimmune polyendocrinopathy–candiasis–ectodermal dystrophy–like autoimmune disease. J. Immunol. 2018, 201, 874–887.

58. Rumi, M.A.K.; Dhakal, P.; Kubota, K.; Chakraborty, D.; Lei, T.; Larson, M.A.; Wolfe, M.W.; Roby, K.F.; Vivian, J.L.; Soares, M.J. Generation of Esr1-knockout rats using zinc finger nuclease-mediated genome editing. Endocrinology 2014, 155, 1991–1999.

59. Goto, T.; Hara, H.; Nakauchi, H.; Hochi, S.; Hirabayashi, M. Hypomorphic phenotype of Foxn1 gene-modified rats by CRISPR/Cas9 system. Transgenic Res. 2016, 25, 533–544.

60. Liang, C.; Zhao, J.; Lu, J.; Zhang, Y.; Ma, X.; Shang, X.; Li, Y.; Ma, X.; Liu, M.; Wang, X. Development and characterization of MDR1 (Mdr1a/b) CRISPR/Cas9 knockout rat model. Drug Metab. Dispos. 2019, 47, 71–79.