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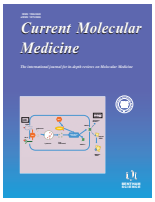
REVIEW ARTICLE

Human Disease Modelling Techniques: Current Progress



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Abstract: Advances in genetic engineering and genomic studies facilitated the development of animal models of human diseases. To date, numerous models based on different animal species are available for the most socially significant human diseases, such as cardiovascular disorders, cancer, neurodegenerative and metabolic disorders. Modern genetic methods allow creating animals with certain genes up- or down-regulated, as well as bearing specific mutations. However, this precision is not easy to translate into clinical practice: animal models still have their limitations, including both physiological and genetic differences between humans and animals and complexity of disease conditions that are difficult to reproduce. In this review, we will discuss the most relevant modern techniques that allow creating genetically engineered animal models.

Keywords: Gene editing, genetic engineering, animal model, CRISPR, human disease, Chromosome 1.

1. INTRODUCTION

Animal models are invaluable for studying human diseases and identifying and testing new therapies. The development of genetic engineering techniques facilitated the development of disease models in various animal species. Still establishing an adequate model that can deliver results translatable to clinical studies remains challenging. For instance, model animals may have a very different lifespan than humans, animal groups may be homogenic in contrast to patient populations that are usually diverse, some experiments may include only male or female animals, which hinders translation of the obtained results to human studies. Finally, the selected disease induction method may turn to be inadequate [1].

A successful animal model should meet a number of criteria that have been formulated based on the accumulated experience: genetic similarity to the corresponding human disease, pathological fidelity, and the way the model responds to existing drugs [2]. The latter is the most important aspect of a preclinical disease model. The importance of correct animal model selection is highlighted by the fact that, in some situations, using different models even based on the same species, can lead to very different conclusions [3].

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Advances in genetics and gene engineering opened infinite possibilities for targeted changes in the animal genome to induce various pathologies. For many laboratory species, the accumulated genetic and phenotypic data were assembled in databases for future reference. For instance, Rat Genome Database (RGD), which has been the primary source of genomic, genetic and phenotype data for rat and mouse since 1999 [4]. RGD provides a set of useful tools for researchers, such as Disease Portals, Ontology browser, Object List Generator and Analyzer. Another example is the Zebrafish All Genes KO Consortium for Chromosome 1 (ZAKOC) project established in China in 2013 [5]. In this review, we will discuss the tools available for making transgenic animal models and will provide examples of animal models the most common human diseases.

2. GENETIC TOOLBOX FOR DEVELOPMENT OF ANIMAL DISEASE MODELS

To date, significant progress has been made in genetic engineering techniques, and numerous methods are now available for designing animal models of human pathologies. While early techniques are still widely used, several new approaches allow for more sophisticated manipulations with animal genome. The most promising methods involve zinc finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeat/ CRISPR-associated protein 9 (CRISPR/Cas9) system.

ZFNs are synthetic restriction enzymes carrying an array of engineered sequence-specific zinc finger DNA binding units linked to the nuclease domain of the restriction enzyme Fok1. The first successful gene editing in rats was achieved due to ZFNs [6]. Successful genetic modification with this method depends on two requirements: an allele with key coding exons flanked by *loxP* sites (floxed allele) and controlled Cre recombinase expression [7]. This technique, which theoretically is applicable for various species, allows targeting two genes located on the same or two different chromosomes.

TALENs have a basic structure similar to that of ZFNs, where the Fok1 nuclease domain is linked to an array of nucleotide-specific DNA binding domains (TAL effectors) and promotes the formation of a Fok1 dimer and the cleavage of the spacer DNA between the TALEN binding sites. With this method, the experiment design is usually simpler than that required for ZFNs. This makes TALENs more suitable for most molecular biology laboratories as a routine gene editing approach [6].

CRISPR/Cas9 system involves Cas9, a riboprotein guided by RNA, that cleaves genomic DNA at designated sites. CRISPR repeats were first discovered in bacteria and Archaea that used them as a functional analogue of the immune system. The system defends microorganisms from viral infections by recognizing foreign DNA previously incorporated within CRISPR and cutting it using Cas9 nuclease (CRISPR-associated protein 9) [8-10]. Genetically engineered CRISPR/Cas9 system can introduce breaks into target DNA to create knock-outs or knock-ins. It would not be an overestimation to say that CRISPR/Cas9 has revolutionized bioengineering. However, this method also has limitations. Some of these limitations can be overcome, such as the simultaneous induction of mutations in multiple target loci. Recently, it became possible to create transgenic Cas9-expressing mice using pronuclear DNA injections [11]. CRISPR methodology combined with somatic cell nuclear transfer approach may be used to create genetically engineered pig models with the desired genetic background for biomedical research [12]. The proposed method has two steps: nuclear transfer when fibroblasts are introduced into enucleated oocytes, and microinjection when CRISPR reagents are injected into cloned embryos. Microinjection of nucleases into one-cell embryos, unlike the conventionally engineered embryonic stem cells, enables instant germline modifications, and accelerates the generation of *in vivo* rodent models for studying human diseases [13].

Thus, TALENs and CRISPR/Cas have a significant potential for creating human diseases models and gene correction under pathologic conditions. Moreover, CRISPR/Cas9 system is an interesting alternative to somatic cell nuclear transfer (SCNT), when generating genetically modified pig and other large animal models [14, 15]. SCNT can cause prenatal or postnatal death and/or reduced efficiency of offspring production due to incomplete epigenetic reprogramming, while gene editing by electroporation of Cas9 protein can overcome this limitation [16].

3. APPLICATIONS FOR BIOMEDICAL RESEARCH

3.1. SCNT and DNA Microinjections

Genetically modified mice and rats remain the most common models for studying human diseases, although there are other animals that are more similar to humans in terms of genetics and physiology, such as pigs. To date, two pig models, N208-105Q and N548-145Q, are the most promising genetically modified pigs used for studying human neurological diseases, such as Huntington Disease. N208-105Q pigs are created by SCNT, via transfecting Tibetan miniature pig fibroblasts with a transgene expressing the N-terminus 208 of human huntingtin (HTT) protein with 105Q (N208-105Q). N548-145Q model is generated by transduction of zygotes with lentiviral vectors expressing an N-terminal truncated form of human HTT (first 548 amino acids) including 145Q (N548-145Q). Spinal muscular atrophy (SMA) can be modeled using single-stranded survival motor neuron gene (SMN)-targeting DNA injection [20]. However, there are still no valid genetically modified porcine Parkinson's Disease and Alzheimer Disease models [16, 17].

In 2015 researchers from The Medical College of Wisconsin in the US provided detailed recommendations for modelling human cardiovascular diseases in rats. According to the guidelines, there are several steps for developing a valid model, such as using phenotypic profiles to identify the strain background, using genomic and transcriptomic data to identify the desired allele, choosing an appropriate technique. Vast knowledge of rat genome allows using a wide array of methods to create genetically modified rats for cardiovascular diseases research site-specific nucleases, transgenesis and using embryonic stem cells. It is also important to choose a suitable genetic background, which will determine the resulting phenotype and will affect the interpretation of results [18]. Animal models for atherosclerosis have limitations, and generally mice and rats are not the best models to represent human condition due to their atherogenesis resistance [19]. But it is possible to create genetically modified models that mimic important aspects of atherosclerosis: fatty streaks, deposition of foam cells, vulnerable and stable plaques, and related complications such as arterial calcification, ulceration, hemorrhage, plaque rupture, thrombosis, and stenosis. Traditional pronuclear microinjection of DNA for producing transgenic rabbit models has average efficiency of 10% and will probably be replaced by knock-outs created with genome-editing techniques, for with the average rate of gene targeting is about 50% [20]. Several genetically modified pig models of cardiovascular diseases have been created as well [21, 22]. These include human CD39 transgenic pigs, peroxisome proliferator-activated receptor gamma (PPARγ) knock-out pigs, low-density lipoprotein

receptor (LDLR) mono-allelic and bi-allelic knock-outs and Niemann-Pick C1-like 1 (Npc1l1) bi-allelic knock-outs that can provide new information about the influence of *Npc1l1* on cardiovascular and metabolic diseases in humans.

Genetically engineered mouse models of cancer have one major advantage over the more common alternative, immunocompromised mice, which is maintaining model animal immune system intact. This leads to a better understanding of interactions between tumor microenvironment and drugs [2, 23]. Mouse models of sporadic cancers can be created by microinjection of DNA in the pronuclei of fertilized zygotes. Such models as mouse model of human familial adenomatous polyposis despite being useful for drug discovery and translational biology have some major disadvantages: this kind of genetic manipulation can result in unexpected phenotypes and uncontrolled variations of levels and patterns of gene expression [24].

3.2. Cre Recombinase-mediated ZFN

In oncology, two types of pancreatic cancer can be studied using genetically engineered animal models: human pancreatic intraepithelial neoplasia (PanINs) and pancreatic ductal adenocarcinoma (PDAC). PanINs can be generated in mice by targeted expression of activated endogenous *KRAS* oncogene in pancreatic progenitor cells using Pdx1 or p48 promotor-driven Cre-mediated recombination (LOX-Stop-LOX), which results in invasive and metastatic carcinoma of human type. Mutations of *Trp53* and *INK4A/ARF* result in development of PDAC features. While new and more advanced models emerge, such as *Ela-K-ras, Mist1-K-ras*, *KrasLSL- G12D/p; DPC4flox/p.*, data they provide has not yet yielded translational implications [25]. Genetically engineered mouse models of colorectal cancer currently used in biomedical research are based on inactivating mutations of the *APC* gene, which underlie familial adenomatous polyposis and can be divided into two groups: truncating *Apc* mutation and Cre-mediated recombination [26]. For bladder cancer, genetically engineered animal model development is still in its early stages. Few such models exist, since relatively few promoters display bladder-specific expression [38]. Developing invasive bladder cancer with prevalent metastasis by delivering of Adeno-Cre system directly into the bladder lumen to inactivate *Trp53* and *Pten* in the urothelium allowed this model to be used in several preclinical studies [27]. However, genetically modeled bladder cancer is less heterogenic, than the human one, and the created models are usually not applicable for testing of novel therapeutic or preventive strategies [28].

Gene silencing or overexpression can be used in combination with other modelling methods for fibrosis. One of the most important aspects of hepatic fibrosis involves hepatic stellate cells functionality (HSCs), therefore gene expression modulation in these cells may provide insight for developing diagnostic and therapeutic approaches. Using the Cre recombinase system controlled by the glial fibrillary acid protein (GFAP) promoter (GFAP-Cre) proved useful in studying the role of autophagy in HSCs during fibrogenesis, gene targets being autophagy-related protein 7 (*ATG7*) gene and *p53* [29]. However, GFAP expression is also detected in cholangiocytes, which limits the application of this approach. A transgenic mouse model involving mice expressing the Cre recombinase controlled by the lecithin-retinol acyltransferase (*Lrat*) promoter crossed with mice expressing ZsGreen Cre reporter can be used for HSCs mapping. Since the expression of *Lrat* is restricted to HSCs and undetectable in other liver cell types, the system allows for specific tagging of 99% of HSCs, that led to confirmation that portal myofibroblasts and activated HSCs are two distinct cell populations [21].

3.3. Viral Delivery of Transgenes

Modelling of breast cancer usually involves expression of transgenes in the mammary epithelium and selective expression of key oncogenes. Mammary gland-specific promoters are MMTV-LTR (mouse mammary tumor virus), WAP (whey acidic protein promoter), BLG (β-lactoglobulin), and K14 [30]. Polyoma middle T antigen (PyMT) is associated with the development of lung and lymph node metastases, thus making MMTV-PyMT the most efficient metastatic model to date. Moreover, it is also possible to create models for studying specific stages of breast cancer, such as ductal carcinoma *in situ* (DCIS) [31]. The development of serial transplantation technique allowed creating a transplantable mouse model, that recapitulated human DCIS and its progression at both histologic and molecular levels. The authors chose 8w-B mouse line, one of the six transplantable mammary intraepithelial neoplasia outgrowth (MIN-O) lines, because of its relatively short tumor latency (11 weeks) and uniform histopathology. The 8w-B MIN-O mice expressed mammary-specific polyomavirus middle T transgene (PyV-mT). Model tumors with a basal phenotype (*C3(1)/Tag, p53−/−*, and *BRCA1−/−*; *p53+/−*) are the best choice to date to model human basal-like breast cancer subtype; MMTV- myc, -ras, -PyMT, and -her2/neu models correlate with luminal B/C subtypes; *p53* mammary epithelial conditional knock-out model correlates with luminal subtype A. Luminal subtypes comprise tumors that express estrogen receptor (ER), while tumors lacking ER expression are either *Her2/Neu* over-expressing ("Her2+") or *Her2* non- expressing ("basal-like") groups. First large-scale miRNA gene expression study across a variety of genetically engineered mouse models of human breast cancer was carried out by Zhu et al., in which both basal and luminal human breast cancer subtypes were represented by eight models: C3(1)/SV40 T/t-antigens, $p53^{fl/fl}$;MMTV-Cre transplant, $BRCA1^{fl/fl};p53^{+/-}$;MMTV-Cre, MMTV-H-Ras, MMTV-*Her2/neu*, MMTV-*c-Myc*, MMTV-PyMT, MMTV-*Wnt1* <μ [32]. The results of that study suggested that tumor lineage determines miRNA expression profiles. Several transgenic animal models have been created to study cancer metastasis. Proto-

oncogene *ERBB2* (also known as *HER2* and the rat homologue, *Neu*) belongs to the EGFR family and encodes a receptor tyrosine kinase. Overexpressing *Neu* in animal models grants tumor cells growth boost, therefore facilitating their migration. Activated form of rat *Neu* under the control of the MMTV promoter with tetracycline regulatory element (MMTV- rtTA/TetO-NeuNT) is one of the examples of murine models of metastasis. Another example is the expression of human *EGFR* under the transcriptional regulation of MMTV or both the MMTV and BLG promoters. MMTV-PyVmT transgenic models that were mentioned earlier are characterized by a far greater metastasis incidence as compared to other MMTV-oncogene expressing transgenic mice. Wnt transgenic models feature *Wnt-1* expressed under MMTV promoter [33]. Astrocytomas, tumors of the central nervous system originating from astrocytes or astrocyte precursor cells, account for almost 40% of all brain tumors. Until now, genetically engineered animal models were less successful for studying astrocytomas due to a number of obstacles, such as resistance modifiers for astrocytoma mouse strain backgrounds, mortality from other tumor types before astrocytoma can develop, species-specific differences between mouse and human. Nevertheless, potential models to study human astrocytomas have been developed: $p19^{ARF}$ null mice, GFAPR/T Ag transgenic mice, GFAP/v-src transgenic mice, RCAS-EGFR; Ntv-a; INK4a−/+mice, RCAS-Kras; RCAS-Akt; Ntv-a mice, Nf1;p53 $cis^{-/+}$ mice [34]. Conditional models of astrocytoma can be of great help for developing and testing new drugs [35]. Animal models greatly contributed to study of angiogenesis, which is one of the rate-limiting steps in tumor growth. RIP-Tag2 genetically modified mice helped estimating the effects of several angiogenesis inhibitors at different stages of carcinogenesis and studying resistance of late-stage PNET to VEGFR2 targeting antibodies, which appeared to be mediated by hypoxia-induced FGF family of proangiogenic ligands [36]. Moreover, transgenic mice expressing human vascular endothelial growth factor (VEGF) gene under the transcriptional control of the MMTV promoter (MMTV-VEGF) crossed with MMTV- PyVmT mice proved to be much more accurate model than “single” transgenic mice, with VEGF contributing to mammary tumor growth by promoting neovascularization, autocrine stimulation of growth, and inhibition of apoptosis [33].

Pig model expressing a dominant-negative gastric inhibitory polypeptide receptor (GIP) receptor (GIPRdn) in pancreatic islets generated by lentiviral transgenesis was the first genetically modified pig model of Type 2 diabetes, which was characterized by significantly reduced oral glucose tolerance, reduced β-cell proliferation and progressive deterioration of glucose control and a reduced pancreatic β-cell mass as they aged [21].

3.4. CRISPR/Cas9

Two approaches to modeling human pathologies using CRISPR/Cas9 are possible: genetic knock-out or knock-in. Gene knock-out using CRISPR/Cas9 involves developing CRISPR guide region, which is to identify target gene region, by creating a single guide RNA (sgRNA), which then directs Cas9 to a specific genomic region where the DNA break is introduced. Then, dsDNA break repair by the non-homologous end joining is initiated in brain, followed by insertion/deletion mutations [37]. Gene expression patterns in the brain are highly dynamic and may vary in different regions, which complicates gene editing [38, 39]. Knock-in mutations are typically introduced via homology-dependent repair (HDR) pathway, which requires a homologous region nearby to a break site, which serves as a template during the repair. The reliance on this pathway is the main limitation of using CRISPR/Cas9 system in neuroscience research [37].

Successful editing of immunoglobulin (Ig) genes in human and mouse B cells by CRISPR-Cas9 technology was recently reported, which can also be used for studying B-cell malignancies [40] Other types of cancer, including lung and pancreatic cancers, are reportedly can be modeled with CRISPR/Cas9 [41]. CRISPR/Cas9 editing capabilities in pospective can be used for creating genetically engineered animal models of cancer with patient-relevant alleles. These techniques include using embryonic stem cells for rapid introduction of additional genetic modifications, reversible silencing of gene expression without modifying the genome via RNA interference [42].

Given that CRISPR/Cas9 method is still relatively new, it is not commonly used for biomedical research, although relevant models of several cardiovascular and infectious diseases do exist [41].

4. FUTURE DIRECTIONS

Personalized medicine strategies based on data obtained from animal models of human pathologies appear very promising, especially for cancer treatment. Recently, two interesting concepts of creating animal models of human cancer were proposed. “Mouse avatars” (or personalized mouse models or patient-derived tumor xenografts models (PDTX)) initiative is considered to be mean to provide information on a specific case of tumorigenesis, which will enable the selection of more effective chemotherapeutic agents. Creation of such models involves transplantation of a portion of a patient’s tumor, obtained either by surgical resection or by biopsy, into immunodeficient mice. Another concept is testing of new drugs for particular cancer in the clinic and in all animal models developed for that cancer simultaneously, which theoretically allows real-time integration of murine and human trial data in an attempt to improve clinical decisions and outcomes. Both concepts are still in development, and authors, though aware of challenges and limitations, see the potential to advance the personalized medicine for cancer treatment [42].

In general, in order to revolutionize biomedical research in future, it is crucial to achieve maximum level of safety and efficiency in genome editing

techniques relevant today before implementing them in development of novel model systems of human pathologies, which is still relatively low for all described here approaches.

CONCLUSION

During the recent years, advances in gene editing techniques opened interesting possibilities for creating more accurate animal models of human diseases. Accumulated knowledge coming from human and animal genomic studies allowed creating extensive databases of relevant mutations that can be used to creating models of various pathologies depending on genetic alterations. Nevertheless, the available models have their limitations, and the translations of results obtained with these models into clinical studies are not straightforward.

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CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Bart van der Worp H, Howells DW, Sena ES, Porritt MJ, Rewell S, O'Collins V, *et al.* Can animal models of disease reliably inform human studies? PLoS Med 2010; 7: 1-8.

[2] Olive KP, Politi K. Translational therapeutics in genetically engineered mouse models of cancer. Cold Spring Harb Protoc 2014; 131-143.

[3] Eisman JA, Bouillon R. Vitamin D: direct effects of vitamin D metabolites on bone: lessons from genetically modified mice. Bonekey Rep 2014; 3: 499.

[4] Shimoyama M, Laulederkind SJF, De Pons J, Nigam R, Smith JR, Tutaj M, *et al.* Exploring human disease using the Rat Genome Database. Dis Model Mech 2016; 9: 1089-1095.

[5] Ma D, Liu F. Genome Editing and Its Applications in Model Organisms. Genomics Proteomics Bioinforma 2015; 13: 336-344.

[6] Meek S, Mashimo T, Burdon T. From engineering to editing the rat genome. Mamm Genome 2017; 28: 302-314.

[7] Brown AJ, Fisher DA, Kouranova E, McCoy A, Forbes K, Wu Y, *et al.* Whole-rat conditional gene knockout via genome editing. Nat Methods 2013; 10: 638-640.

[8] Bortesi L, Zhu C, Zischewski J, Perez L, Bassié L, Nadi R, *et al.* Patterns of CRISPR/Cas9 activity in plants, animals and microbes. Plant Biotechnol J 2016; 14: 2203-2216.

[9] Carroll D. Genome Editing: Past, Present, and Future . Yale J Biol Med 2017; 90: 653-659.

[10] Tycko J, Myer VE, Hsu PD. Methods for Optimizing CRISPR-Cas9 Genome Editing Specificity. Mol Cell 2016; 63: 355-370.

[11] Sakurai T, Kamiyoshi A, Kawate H, Mori C, Watanabe S, Tanaka M, *et al.* A non-inheritable maternal Cas9-based multiple-gene editing system in mice. Sci Rep 2016; 6: 20011.

[12] Sheets TP, Park CH, Park KE, Powell A, Donovan DM, Telugu BP. Somatic cell nuclear transfer followed by CRIPSR/Cas9 microinjection results in highly efficient genome editing in cloned pigs. Int J Mol Sci. 2016; 17.

[13] Cai M, Yang Y. Targeted Genome Editing Tools for Disease Modeling and Gene Therapy. Curr Gene Ther 2014; 14: 2-9.

[14] Crispo M, Mulet AP, Tesson L, Barrera N, Cuadro F, Dos Santos-Neto PC, *et al.* Efficient generation of myostatin knock-out sheep using CRISPR/Cas9 technology and microinjection into zygotes. PLoS One 2015; 10: e0136690.

[15] Tang L, Gonzales R, Dobrinsk I. Germline modification of domestic animals. Anim Reprod 2015; 12: 93-104.

[16] Holm IE, Alstrup AKO, Luo Y. Genetically modified pig models for neurodegenerative disorders. J Pathol 2016; 238: 267-287.

[17] Fan N, Lai L. Genetically Modified Pig Models for Human Diseases. J Genet Genomics 2013; 40: 67-73.

[18] Flister MJ, Prokop JW, Lazar J, Shimoyama M, Dwinell M, Geurts A. 2015 Guidelines for Establishing Genetically Modified Rat Models for Cardiovascular Research. J Cardiovasc Transl Res 2015; 8: 269-277.

[19] Getz GS, Reardon CA. "Animal Models of Atherosclerosis," in Animal Models for the Study of Human Disease: Second Edition (Baishideng Publishing Group Inc) 2017; 205-217.

[20] Fan J, Kitajima S, Watanabe T, Xu J, Zhang J, Liu E, *et al.* Rabbit models for the study of human atherosclerosis: From pathophysiological mechanisms to translational medicine. Pharmacol Ther 2015; 146: 104-119.

[21] Yao J, Huang J, Zhao J. Genome editing revolutionize the creation of genetically modified pigs for modeling human diseases. Hum Genet 2016; 135: 1093-1105.

[22] Yum SY, Yoon KY, Lee C Il, Lee BC, Jang G. Transgenesis for pig models. J Vet Sci 2016; 17: 261-268.

[23] Becher OJ, Holland EC. Genetically engineered models have advantages over xenografts for preclinical studies. Cancer Res 2006; 66: 3355-3358.

[24] Cekanova M, Rathore K. Animal models and therapeutic molecular targets of cancer: Utility and limitations. Drug Des Devel Ther 2014; 8: 1911-1922.

[25] Mohammed A, Janakiram NB, Lightfoot S, Gali H, Vibhudutta A, Rao CV. Early detection and prevention of pancreatic cancer: use of genetically engineered mouse models and advanced imaging technologies. Curr Med Chem 2012; 19: 3701-13.

[26] Roper J, Hung KE. Priceless GEMMs: Genetically engineered mouse models for colorectal cancer drug development. Trends Pharmacol Sci 2012; 33: 449-455.

[27] Kobayashi T, Owczarek TB, McKiernan JM, Abate-Shen C. Modelling bladder cancer in mice: Opportunities and challenges. Nat Rev Cancer 2015; 15: 42-54.

[28] Ding J, Xu D, Pan C, Ye M, Kang J, Bai Q, *et al.* Current animal models of bladder cancer: Awareness of translatability (Review). Exp Ther Med 2014; 8: 691-699.

[29] Delire B, Stärkel P, Leclercq I. Animal Models for Fibrotic Liver Diseases: What We Have, What We Need, and What Is under Development. J Clin Transl Hepatol 2015; 3: 53-66.

[30] Greenow KR, Smalley MJ. Overview of Genetically Engineered Mouse Models of Breast Cancer Used in Translational Biology and Drug Development. Curr Protoc Pharmacol 2015; 70: 14.36.1-14.36.14.

[31] Namba R, Maglione JE, Young LJT, Borowsky AD, Cardiff RD, MacLeod CL, *et al.* Molecular characterization of the transition to malignancy in a genetically engineered mouse-based model of ductal carcinoma in situ. Mol Cancer Res 2004; 2: 453-463.

[32] Zhu M, Yi M, Kim CH, Deng C, Li Y, Medina D, *et al.* Integrated miRNA and mRNA expression profiling of mouse mammary tumor models identifies miRNA signatures associated with mammary tumor lineage. Genome Biol 2011; 12: R77.

[33] Menezes ME, Das SK, Emdad L, Windle JJ, Wang XY, Sarkar D, *et al.* Genetically engineered mice as experimental

tools to dissect the critical events in breast cancer. Adv Cancer Res 2014; 121: 331-382.
[34] Reilly KM, Jacks T. Genetically engineered mouse models of astrocytoma: GEMs in the rough? Semin Cancer Biol 2001; 11: 177-190.
[35] McNeill RS, Vitucci M, Wu J, Miller CR. Contemporary murine models in preclinical astrocytoma drug development. Neuro Oncol 2015; 17: 12-28.
[36] van Miltenburg MH, Jonkers J. Using genetically engineered mouse models to validate candidate cancer genes and test new therapeutic approaches. Curr Opin Genet Dev 2012; 22: 21-27.
[37] Walters BJ, Azam AB, Gillon CJ, Josselyn SA, Zovkic IB. Advanced in vivo use of CRISPR/Cas9 and anti-sense DNA inhibition for gene manipulation in the brain. Front Genet 2016; 6: 362.
[38] Goldman SM, Kamel F, Ross GW, Bhudhikanok GS, Hoppin JA, Korell M, *et al*. Genetic modification of the association of paraquat and Parkinson's disease. Mov Disord 2012; 27: 1652-8.
[39] Jiao Y, Lu L, Williams RW, Smeyne RJ. Genetic dissection of strain dependent paraquat-induced neurodegeneration in the substantia nigra pars compacta. PLoS One 2012; 7: e29447.
[40] Cheong T-C, Compagno M, Chiarle R. Editing of mouse and human immunoglobulin genes by CRISPR-Cas9 system. Nat Commun 2016; 7 : 10934.
[41] Martinez-Lage M, *et al*. CRISPR/Cas9 Technology: Applications and Human Disease Modeling. Progress in Molecular Biology and Translational Science 2017; 152: 23-48.
[42] Malaney P, Nicosia SV, Davé V. One mouse, one patient paradigm: New avatars of personalized cancer therapy. Cancer Lett 2014; 344: 1-12.
[43] Tanihara F, Takemoto T, Kitagawa E, Rao S, Thi L, Do K, *et al*. Somatic cell reprogramming-free generation of genetically modified pigs. Sci Adv 2016; 2: 1-9.
[44] Kersten K, de Visser KE, van Miltenburg MH, Jonkers J. Genetically engineered mouse models in oncology research and cancer medicine. EMBO Mol Med 2017; 9: 137-153.
[45] Leong XF, Ng CY, Jaarin K. Animal Models in Cardiovascular Research: Hypertension and Atherosclerosis. Biomed Res Int 2015; 528757.