

A booming field of large animal model research

Animal models are integral to the study of fundamental biological processes and the etiology of human diseases. Small animal models, especially those involving mice, have yielded abundant and significant insights, greatly enhancing our understanding of biological phenomena and disease mechanisms. The preference for small animal models is primarily due to their ease of use and the availability of well-established genetic manipulation tools. The extensive use of genetically modified small animals has led to remarkable advancements in biomedical research. Nevertheless, it is important to recognize the substantial disparities in genomes, anatomy, and physiology between humans and small animals. As a result, there is an increasing awareness of the need to utilize large animal models that more closely resemble humans. Such models are essential for enhancing our understanding of crucial biological issues as well as the pathogenesis of diseases.

This special column about “*Large Animal Model Research*”, together with a previous review in this journal (Rahman et al., 2023), focusing specifically on large animal models and highlighting the use of various large animal models, including rabbits, pigs, and non-human primates (NHPs), in addressing key biological and medical issues. Incorporating larger animals into research not only substantially enhances the variety of research tools and methodologies available, but also enables the exploration of long-standing issues that have proven challenging using small animals, given their species-specific differences.

Among the abovementioned large animals, rabbits are comparatively less commonly employed as a model system for research. However, the comprehensive review by Han et al. (2024b) offers valuable insights into the utilization of rabbit models in the field of biomedical research. Rabbits represent a cost-effective advantage over larger animals, owing to their ease of handling and rapid reproductive capacity, while also exhibiting larger body sizes and longer lifespans than rodents. These unique attributes, coupled with advancements in genetic modification techniques, such as CRISPR/Cas9 gene targeting, have led to the establishment of various rabbit models for biomedical research. Given that their lipid metabolic profiles and immune responses are more similar to humans than to rodents, rabbits are considered suitable for modeling cardiovascular and immune-related diseases. Furthermore, with their extensive involvement in commercial antibody production, genetically modified rabbits are an asset in the development of antibody-based drugs and

immunotherapeutic agents. Although the generation of rabbit models dedicated to the exploration of neurological disorders has been relatively limited, a recent study using base editing technology to modify the amyotrophic lateral sclerosis (ALS) gene *SOD1* successfully produced a rabbit model exhibiting ALS phenotypes (Zhang et al., 2023). Thus, this study underscores the potential of rabbits in investigating neurological disorders.

Regarding neurological disorder research, NHPs play an indispensable role due to their high similarity to humans in terms of brain structure, function, and aging processes. In their comprehensive review, Pan et al. (2024) discuss several NHP models of neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), ALS, and Huntington's disease (HD). The analysis of these disease models underscores the significance of employing NHPs for the investigation of the fundamental mechanisms underpinning neurodegenerative diseases.

Despite their extensive use over the past few decades, genetically modified mouse models cannot fully replicate the pathological features observed in human patients. A notable discrepancy is the absence of pronounced and selective neuronal loss in most genetically modified mouse models. For instance, mouse models carrying genetic mutations associated with PD do not exhibit degeneration of dopaminergic neurons, and most mouse models of ALS lack significant cytoplasmic accumulation of TDP-43, both of which are key pathological manifestations observed in human PD and ALS, respectively. In contrast, monkeys expressing PD-associated proteins (Li et al., 2021a; Yang et al., 2019) or mutant TDP-43 (Yin et al., 2019) successfully recapitulate neurodegeneration and cytoplasmic TDP-43 accumulation, respectively.

Neurodegenerative features are not limited to primates; they also occur in other types of large animals. Pigs have been utilized in the exploration of neurological disorders due to their genetic, anatomical, and physiological similarities to humans. In the review by Han et al. (2024a), pigs, NHPs, and sheep were compared for their utility in studying HD, a monogenetic disorder with a genetic mutation that can be replicated across different species. Similar to mouse models of AD and PD, mouse models carrying the HD gene also fail to exhibit the robust and overt neurodegeneration observed in afflicted patient brains. Several transgenic large animal models have been established in NHPs, pigs, and sheep. Nevertheless, these models present a spectrum of phenotypic variations, and the progression and severity of disease differ significantly. Given that animal behaviors and phenotypes are strongly influenced by transgene copy number and expression levels, ideal animal models are those that can faithfully recapitulate human genetic mutations and express mutant genes at the endogenous level.

Pigs possess distinct genetic modification advantages compared to NHPs and sheep. Notably, somatic nuclear

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transfer, which allows for the knock-in (KI) of mutant genes, has been successfully applied in pigs to generate a HD gene KI model that recapitulates selective neuronal loss and motor function deficiency, two crucial pathological features of HD. This HD KI pig model has been effectively employed in gene therapy research to reduce HD pathology and symptoms (Yan et al., 2023), providing further evidence for the importance of large animal models in the investigation of neurodegenerative diseases.

Pigs are also extensively used in other biomedical research fields, including circulatory system diseases, organ transplantation, diabetes, skin diseases, and tumors. Porcine models also show promise for studying inherited hearing loss. Notably, pigs have a hearing range comparable to that of humans and naturally undergo age-related hearing loss, a phenomenon largely attributed to the remarkable similarity in both the structure and function of their ears to those of humans. Genetic modification techniques, such as CRISPR/Cas9 gene editing and somatic nuclear transfer, have enabled the creation of single genetic mutations in pig models, facilitating the study of disease pathogenesis in monogenic hearing loss. For example, Wang et al. (2024) discussed the use of pig models in studying hearing loss-related diseases, with notable implications for other monogenic diseases.

The four research articles published in this special issue also highlight the significance of large animal models in research. Li et al. (2024a) reported on mitochondrial replacement in cynomolgus monkeys (*Macaca fascicularis*) through female pronuclear transfer. Their findings suggest that pronuclear transfer holds great potential in reducing the risk of inherited mitochondrial DNA (mtDNA) diseases, thereby establishing the applicability of non-human models in investigating diseases related to mtDNA defects. Li et al. (2024b) developed cynomolgus monkey organoids to study neural tube defects (NTDs), which can also arise from loss-of-function mutations in the *SHROOM3* gene. Their findings indicate that *in vitro* models using NHP-derived organoids can be employed to investigate the pathogenesis of significant human diseases.

The rationale for using NHPs in research is also based on species-dependent gene expression patterns. Chen et al. (2024) presented intriguing findings regarding the expression of *PINK1*, which is associated with PD, in mice, pigs, and monkeys. Using multiple antibodies and *PINK1* knockout animal models, they discovered that the *PINK1* protein, rather than *PINK1* mRNA, is detectably and exclusively expressed in the primate brain. These findings explain why *PINK1* knockout leads to neurodegeneration in monkeys, but not in mice and pigs, suggesting that a possible higher abundance of endogenous *PINK1* in primate brains contributes to neuronal survival. Their results also imply that species-dependent transcriptional and translational regulations contribute to species-specific pathology. Therefore, utilizing animal models that more closely resemble humans provides better prospects for uncovering the molecular mechanisms underlying primate-specific gene expression. Reinforcing this idea, Mao et al. (2024) conducted a comprehensive analysis of the transcriptomes within macaque species and between macaques and humans, highlighting the high conservation of tissue-specific genes and the value of macaques as biological models for investigating human diseases. Additionally, the identification of a cynomolgus monkey with naturally occurring

PD further supports the suitability of NHPs as ideal models for PD research (Li et al., 2021b).

Despite the significant contributions and advancements in large animal models for biomedical research, notable limitations and challenges exist. The utilization of large animals necessitates stricter regulation and control due to ethical concerns arising from their high cognitive capacities and close resemblance to humans. Additionally, the substantial costs and extended breeding periods associated with maintaining large animals pose considerable challenges in expanding the scope of studies using these models. Undoubtedly, small animal models, particularly rodents, are likely to remain fundamental in biological and biomedical research, continuing to play a critical role in enhancing our understanding of disease pathogenesis and in developing therapeutic strategies for human diseases. However, in scenarios where small animal models have failed to replicate important pathological events observed in humans, it is worth considering large animal alternatives to unravel disease mechanisms that may not be evident in small animals. Furthermore, large animal models can facilitate the validation of specific therapeutic targets, potentially reducing the failure rates of clinical trials for therapeutics initially developed using small animal models. The integration of large animal models into research endeavors promises to deepen our understanding of complex biological processes and improve the translation of preclinical findings into clinical practice.

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