RESEARCH HIGHLIGHT

Using Huntingtin Knock-In Minipigs to Fill the Gap Between Mouse Models and Patients with Huntington's Disease

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Huntington's disease (HD) is an inherited autosomal dominant neurodegenerative disease characterized by progressive motor deficits, cognitive decline, and psychiatric symptoms. It is caused by a pathological expansion of CAG trinucleotide repeats in exon 1 of the HD gene, resulting in the translation of a mutant form of huntingtin protein (mutant Htt) with an expanded polyglutamine domain in the N-terminal region [1]. Despite great progress in understanding the pathogenesis of HD using multiple mouse models, the exact mechanisms by which mutant Htt induces neuronal dysfunction and death are still not completely clear, and there is no curative treatment for this disease. An important reason is that the mouse, which is the most widely used animal model in HD research, differs from the human in many aspects, including the physiology, drug metabolism, blood-brain barrier, life span, brain volume, and neuroanatomical organization [2]. Thus, it is necessary to establish HD models with higher species than rodents, such as the dog, pig, and non-human primate, so as to bridge the gap between preclinical mouse models and clinical studies.

Pigs share many similarities with humans, including dietary structure, body size, anatomy, physiology, biochemistry, and metabolism, and suffer from the same genetic and protein malfunctions linked to many human

diseases, making them important biomedical models for studying the pathogenesis and therapeutic strategies of human diseases [3, 4]. Recently, Yan *et al.* used the CRISPR/Cas9 gene-editing system to introduce a segment of a human gene encoding mutant Htt containing 150 glutamine repeats into fetal pig fibroblasts, and then used somatic cell nuclear transfer to generate an HD knock-in minipig model [5]. This model expresses full-length mutant Htt at the endogenous level and exhibits the symptoms of human HD including movement problems, behavioral abnormalities, and respiratory difficulties, along with striking and selective degeneration and death of medium spiny neurons in the striatum—the brain region vulnerable in HD. In addition, the neuropathological changes and disease phenotypes are transmissible *via* germline cells. These findings suggest that the minipig model has more advantages in mimicking human HD than mouse models (Fig. 1). Even though the mouse models replicate many of the clinical and molecular events of HD, they do not show the respiratory difficulty phenotype and the robust neuronal cell loss in affected brain areas that is the major neuropathological hallmark in HD patients. Furthermore, increasing evidence has shown that neuronal and synaptic dysfunction precedes cell death by many years in the brain of HD patients, and HD has been suggested to be a synaptopathy [6–8]. The HD knock-in pig may be a better model in which to uncover the disease progress from the early stage of synaptic abnormalities to the later stage of neuronal cell loss and its molecular mechanisms and therapeutic targets.

Although nonhuman primates are considered as the animal model gold standard in preclinical research, the minipig is becoming a routine model in experimental and translational medicine due to its advantages in terms of ethical considerations, cost, availability, housing,

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Fig. 1 Comparison of the advantages in mouse and mini-pig HD models.

experimental manipulation, breeding period, and litter size [9, 10].

Of course, the HD knock-in minipig has its shortcomings. First, like most of the HD mouse models, this minipig model expresses a mutant Htt containing a large polyglutamine repeat and usually represents a juvenile-onset form of HD. However, only ~ 6% of HD patients have juvenile HD, most having the adult-onset form [11]. Since the clinical manifestations of juvenile HD are very different from those of adult-onset HD, caution should be exercised in the interpretation of experimental results using this model, and more minipig models with different CAG repeat lengths should be developed for various preclinical studies. Second, although abundant background data on pigs are available, their characteristics vary greatly depending on breed, and only very few published articles refer to using the Rongshui miniature pig in this model. Third, research using minipig models is more expensive and takes more time than mouse models, and many investigators lack the experimental conditions for large-animal studies. In addition, the availability of appropriate kits/reagents/assays for minipigs is limited, and they are not the preferred option if sulfation plays an important role in the human metabolism of the candidate drug [12].

Together, the technology of CRISPR/Cas9 gene editing combined with somatic cell nuclear transfer is very useful and practical for producing a relatively perfect non-rodent mammal model of HD despite the high cost and low success rate. This HD knock-in minipig model recapitulates the clinical features and neuropathological changes seen in HD patients and

provides a stable and reliable tool to bridge the gap between mouse models and HD patients by validating the mechanisms and therapeutic targets found in the mouse models. It will also provide new clues for HD pathogenesis and treatment.

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