The Rise of Cloned Monkeys for Biomedical Studies

Reported by YAN Fusheng

The biological differences between the widely used rodent models and humans have caused many fiascos in pharmaceutical development. Proving drugs effective in rodents does not guarantee they also work for humans. Unfortunately, this is true in most cases. Because of this, animal models that mimic humans more closely are of great value. Non-human primates are considered to be the best animal models, given that they are genetically the closest to humans. Cloning these animals, however, has been a long-standing challenge; many attempts have ended in failure.

Things changed in early 2018. Two live macaque monkeys, *Zhongzhong* and *Huahua*, were successfully cloned by a CAS research team led by Profs. SUN Qiang and LIU Zhen at the **CAS Institute of Neuroscience (ION)/CAS**

The two cloned monkeys ("*Zhongzhong*" and "*Huahua*") have grown up.

Center for Excellence in Brain Science and Intelligent Technology in Shanghai. The work represents a proof-of-the-concept demonstration. In the future, many more identical cloned monkeys could be produced in labs as ideal models for studying human biology and diseases, particularly when they carry a customized disease.

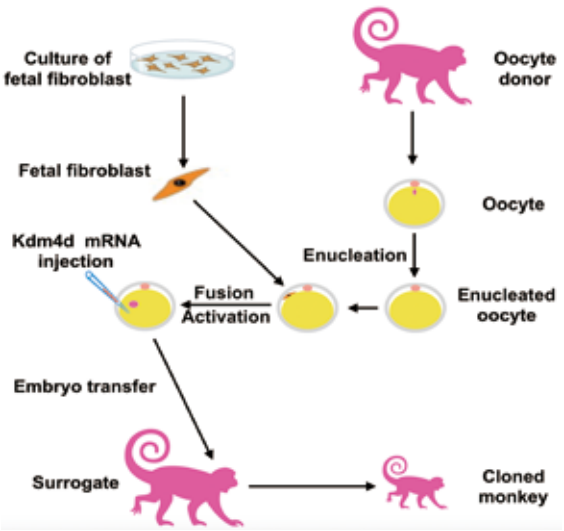
The feat was accomplished via somatic cell nuclear transfer (SCNT), the same method that created Dolly-the-sheep in the 1990s. This method replaces the nucleus of a chosen egg cell (the host) with the one extracted from a differentiated somatic cell (or body cell, the donor). After being inserted into the enucleated egg, the body cell nucleus is somehow "reprogrammed" by its host egg cell to regain the lost ability called "totipotency," the potency to grow into any type of cells that are needed to develop and form an organism. Stimulated with a proper "shock," the fusion cell – the fusion of donor nucleus and enucleated egg cell – starts to divide and gradually forms an embryo that can be transplanted into a surrogate womb, where it can ultimately develop into a full-term fetus.

Previous attempts using adult somatic cells as nuclear donors failed to yield live monkey offspring. The poor reprogramming potency of the adult nuclei was considered to take the blame. To bypass this pitfall, the CAS team chose fetal nuclei as the DNA donor, given that they could be more readily reprogrammed by the host egg cell. Besides, by using fetal fibroblasts as donor cells coupled with *in vitro* screening, researchers can quickly obtain large amounts of identical donor nuclei that have the same gene edits. As a result, many genetically identical monkey models can be quickly produced to meet research needs.

The researchers also made further efforts to improve the reprogramming potency of these transferred fetal nuclei.

In the fetal nuclei, there exist particular genomic regions called reprogramming-resistant regions, where the genes are densely packed and are thus hard to be reprogrammed. It was found that these regions feature an increased level of a certain methyl tag – termed histone 3 lysine 9 trimethylation, or H3K9me3 for short– on the histone cores. The increased level of H3K9me3 tags tightens the DNA package in these regions and thus makes the genes there hard to be reprogrammed. To overcome these reprogramming barriers, the team sought to loosen these densely packaged regions.

Genomic DNA wraps around the histones to be packaged. The tightness of DNA packaging is mainly controlled by chemical tags on the core histones. Chemically



A schematic workflow for creating cloned monkeys via somatic cell nuclear transfer (SCNT), using monkey fetal fibroblasts as nuclear donors to replace egg cell nuclei.

tagging the core histones with methyl or acetyl can, respectively, tighten or loosen the DNA packaging. Therefore, less methylation or more acetylation on the histones can bring the chromosomes to a relatively relaxed or opened state, whereby a chromosome is more accessible for the reprogramming process. In this regard, researchers injected H3K9me3 demethylase *Kdm4d* mRNA at the one-cell stage to reduce these certain methyl tags. Meanwhile, they also treated the fusion cell with histone deacetylase inhibitor trichostatin A to enhance the overall acetylation level on the histones. With this combined treatment, they managed to enhance the reprogramming potency of the transferred DNA. As a result, they achieved an improved success rate and better quality in embryonic development of the fusion cell.

Genetic analysis confirmed that the nuclear DNA of the cloned monkeys was identical to the donor fetal fibroblasts, and the mitochondrial DNA was identical to the egg cell donors. This verifies that the cloning is successful. This success in cloning macaques has broken the technical barriers of cloning non-human primates. It has also created a new era, of using non-human primates as experimental models, and is a truly exciting milestone in biomedical studies.

"In the near future, China may grow into a global hub for biomedical studies and pharmaceutical tests for human diseases," says ION director Prof. POO Muming, a co-author of the study.

Reference

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