Cell Research (2013) 23:1161-1162.

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RESEARCH HIGHLIGHT

The Years of the Monkey

Cell Research (2013) **23**:1161-1162. doi:10.1038/cr.2013.111; published online 20 August 2013

The fact that mammals are diploid sets a barrier to rapidly understand the function of non-coding and coding genes in the genome. Recently, Yang *et al*. reported successful derivation of monkey haploid embryonic stem cells from parthenotes, which provide an effective platform for studying mammalian gene function and enable reverse genetic screening of genes for recessive phenotypes in monkeys.

According to the Zodiac in the Chinese Calendar, the next year of the monkey is not slated until February 2016, but a recent paper in this month's *Cell Research* suggests that it may have arrived early for the field of stem cell biology. In a stunning technical "Tour de Force", Jinsong Li and his colleagues report for the first time the generation of several independent haploid monkey embryonic stem (ES) cell lines [1], building on the previous work from their lab and others that described the generation of murine haploid ES cell lines [2-5] (Figure 1). They first activated metaphase II monkey oocytes with ionomycin followed by cycloheximide treatment. These activated oocytes could develop into blastocysts *in vitro* and haploid ES cells (haESCs) can be derived by culturing the inner cell mass in a standard monkey ES cell culture system and using Hoechst FACS technique. Remarkably, one of the cell lines remained stable during long term passage, obviating the need for FACS sorting for the haploid cell lines during subsequent propagation. The cell lines can be genetically manipulated by insertional mutagenesis or by PiggyBac transposon technology, suggesting the possibility of genome-wide screening strategies. In this regard, a series of parallel scientific advances suggest that this technology platform may be particularly timely as the field of stem cell biology moves towards regenerative medicine and therapeutics.

For many years, it has proven quite difficult to engineer site-specific mutations, knock-ins, and knock-outs in human ES or induced pluripotent stem (iPS) cells, and only a handful of genetically engineered lines have been created by conventional homologous recombination strategies [6]. However, recent advances in RNA-guided nuclease technology has led to a marked improvement in the efficiency of the knockout of genes in human pluripotent stem cells [7], suggesting that it may be possible to create knock-out haploid non-human primate (NHP) ES cell lines that harbor specific disease genes and surrogate reporter readouts, and then to look for genetic complementation that could identify critical genes that could be potential drug targets. A library of individual NHP haploid ES cell lines that harbor a loss-of-function mutation across the entire NHP genome could find multiple uses in quickly identifying signaling pathways in differentiated cell types. Given recent advances in screening in human ES and iPS cell lines [8], direct drug screening on the haploid monkey ES cell lines should also be possible. In addition, it will likely be possible to set up genome-wide screening to systematically identify entire network of genes that drive specific differentiation events, and early steps of primate organogenesis. If androgenetic NHP haploid cell lines can be developed (see Figure 1), a leap in the efficiency of the generation of monkey KO animal models could be envisioned over the long term. In this regard, the recent generation of chimeric monkeys [9], as well as future technical advances related to this achievement, could become of significant interest.

At the same time, the study indirectly raises the query as to the need for monkey model systems when the technology for genetic manipulation in the mouse is without peer, and human ES and iPS cell lines can now be easily generated and genetically manipulated. The recent pronouncement of the termination of NIH support for primate research (http://news.sciencemag.org/people-events/2013/06/nih-will-retire-most-research-chimps-end-many-projects), along with the growing awareness of the need to re-examine the need for NHP models, suggests that there must be very solid scientific grounds for pursuing NHP model systems in the future.

In this regard, a growing body of evidence is now pointing to the lack of fidelity of mouse models of human disease to the *in vivo* human setting, a problem that has plagued cancer therapeutics for decades. Recently, the lack of predictability of human responses from models of murine sepsis has been cogently made [10], and the divergence in the physiology of mice and humans, particularly in terms of metabolism and cardiovascular, are enormous. The complexity and scalability of primate versus murine organogenesis also may be an issue. For example, the human heart is 10 000 times larger than the murine, has a much larger diversity of cell types, and a level of tertiary morphology that is not found in the murine heart (for review see

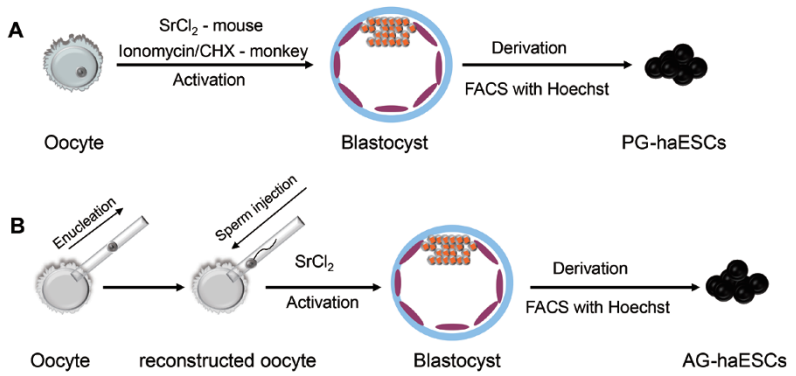

Figure 1 The scheme of parthenogenetic (PG) and androgenetic (AG) haploid embryonic stem cells (haESCs) derivation. **(A)** For the generation of PG-haESCs, metaphase II oocytes were activated with either strontium chloride ($SrCl_2$) for mice or ionomycin/cycloheximide (CHX) for monkeys and further cultivated to the blastocyst stage. With the help of Hoechst FACS technique, PG-haESCs can be derived. **(B)** For the generation of AG-haESCs, metaphase II oocytes were enucleated followed by sperm injection. In addition, the reconstructed oocytes were activated with $SrCl_2$ for mice and further developed to the blastocyst stage *in vitro*. AG-haESCs can be derived by several rounds of Hoechst FACS based on DNA contents. The derivation of non-human primate AG-haESCs has not been reported yet.

[11]). Murine cardiogenesis is largely completed with 48 h, while human cardiogenesis occurs over months, and recent studies that suggest a much larger diversity and markedly extended period of proliferation of the family of heart progenitors in the human fetal versus murine heart [12]. To date, there are no approved drugs that have come from genetically engineered murine models of cardiovascular (CV) disease, and the biggest CV drugs have actually been discovered based on human genetics (statins, PCSK9, *etc*.). The increased importance of CV side effects for new drugs in the diabetes space, as well as for other chronic diseases, points to the importance of their study in more sophisticated primate systems, as all these drugs (Avandia, Vioxx, *etc*.) had cleared conventional screening in rodent model systems. Given the above, we may have to put the Chinese Calendar on auto-repeat mode, as we enter the "Years of the Monkey" in this decade and the next.

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