Commentary

Stem Cell INVESTIGATION

Non-human primate chimeras make a move

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Imagine harvesting individualized human organs for transplantation from animal donors. Recent experimental progress on interspecies chimera has elevated this vision onto a more realistic level Pioneering work by Kobayashi *et al.* used rat pluripotent stem cells (PSC) to complement pancreas-deficient mouse blastocysts for the generation of chimeric interspecies mice with a rat pancreas, and Matsunari *et al.* generated pigs carrying a stem cell derived chimeric pancreas (1,2). However, the interspecies blastocyst complementation method to generate chimera for example from human PSC in a non-human host faces numerous prohibitive hurdles: Besides anatomical differences stands its inefficiency since chimerism across many tissues is highly toxic, and the problem increases with evolutionary distance. Even the evolutionary close rat-mouse chimeras produced by injection of mouse embryonic stem cells (ESC) into rat blastocysts can only accommodate a low degree of chimerism, yet already show morphological abnormalities (3). This observation seems to justify pessimism as to whether animal-human chimerism can be achieved by standard blastocyst complementation. The use of human PSC for the generation of interspecies chimera appeared even more challenging as it was not possible until now to even generate chimeric non-human primates using same species monkey PSC. This challenge has now been tackled in an elegant study by Chen *et al.* (4).

Chen *et al.* first induced transition of epiblastic, or primed, cynomolgus monkey ESC (cESC) into embryonic or ground state—like cells using an adaptation of previously established media (5). The resulting cESC maintained typical features of a naïve phenotype such as dome formation, shortened doubling time and enhanced cloning efficacy over at least 27 passages. Transcriptome analysis revealed typical changes, including activation of LIF-Stat3, PI3K-Akt, Nodal-Smad2/3, and Wnt signaling pathways and increased expression of extracellular matrix proteins. The higher expression in primed cESC of transcripts associated with cell adhesion, cell death, and tight junctions not only indicates that cell-cell interaction are necessary for survival of primed cESC, but together with the upregulation of genes related to the immune system, may also hint at possible reasons for the inability of primed cells to successfully form chimera. Whether some primed ESCs survive post implantation and selectively participate in organ development is currently not known.

The stable induction of cESC resembling a ground state allowed the generation of chimeras in cynomolgus monkeys. Blastocyst complementation with these cells produced chimeric fetuses in 1 of the 5 recipient monkeys. The two fetuses were both developmentally normal with chimerism reaching between 1 and at least 18% depending on organ and included donor-derived germ cell progenitors. Although pregnancy with chimeric fetuses was terminated at the end of the second trimester, it is likely that normal offspring would have been born. Interesting, the authors found a strong impact of media conditions on cESC maintenance after blastocysts injection suggesting a still instable phenotype of the induced naïve cESC. Even after grafting into a supposedly ESC—permissive blastocyst environment, the injected cESC failed to be maintained and survive in medium optimized for *in vitro* blastocyst development. Instead, it provoked rapid differentiation of cESC. In contrast, medium used to induce the naïve—like cESC phenotype promoted cESC maintenance in blastocysts, but at the same time had a negative effect on blastocyst development. It would be important to define

the adjustment kinetics of the injected cells and identify the media components relevant for this counterintuitive effect, and perhaps to test other media conditions used to induce the ground state in human ESC (6,7). In addition, the utilization of the protocol towards the generation of chimera in other primate species in an intra- and interspecies setting would not only verify the data but also be a step towards its broader applicability. To test the different variables to optimize generation of chimerism in non-human primates would require a large number of blastocysts and host animals. A recently developed *in vitro* assay to test and optimize the conditions to establish chimerism by blastocyst complementation, including interspecies chimerism, has recently been developed (8) and may be useful to test culture conditions and cell compatibilities. In this assay, PSC are used to complement host blastocysts, which are then cultured *in vitro* up to the head-fold stage. When naïve-like and conventional human PSC were used in these assays, only few cells survived but never integrated into egg cylinder stage mouse embryos (8).

The results presented by Chen *et al.* not only pave the way towards the systematic assessment of ESC potency in chimeric non-human primates, they moreover establish a cornerstone on the way to application for human medicine. One application aspect would be the modeling of human disease in monkeys via chimeric technology using diseased PSC. The established disease models can then be used for drug and toxicity testing in an *in vivo* environment that more closely resembles the human situation in terms of genetics and metabolism. Eventually it may be possible to generate human-monkey chimera to establish monkeys with a humanized immune system, or with other humanized healthy or diseased organs, which provide advanced models for drug and toxicity testing, but also for mode of action studies of human cell therapies. Whether it is possible to also generate human organs in monkey hosts for harvesting and subsequent use as organ grafts in patients is likely still a long way to go. Human organs may be harvested from fetal monkeys and further maturated either *ex vivo* or post transplantation in the human patient or in a secondary animal host such as pig (9). The work on non-human proximate chimera will certainly provide a model system to study primate PSC interspecies chimera formation also with evolutionary more distant animals.

Finally, it is clear that the progress in this field raises serious ethical risks that need to be discussed and mitigated by establishing a relevant regulatory framework and in parallel by the development of specific animal models that control the degree of humanization (10). The work by Chen and coworkers has shown that the establishment of monkey chimeras is possible and has brought the vision of generating human monkey interspecies chimera another step forward.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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