Generation of embryo chimeras with pluripotent stem cells in non-human primates.

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Poster + Flash-talk

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In contrast to rodent pluripotent stem cells (PSCs), which self-renew in the naïve state of pluripotency, conventional non-human primate PSCs (NHP-PSCs) self-renew in the primed state of pluripotency. As a result, they are unable to colonise pre-implantation embryo to form somatic chimeras. We developed an original strategy to reprogram NHP-PSCs to naïve-like pluripotency using LIF, Activin, and chemical inhibitors of PKC and WNT signalling. The resulting cells, called 2CLA, acquired gene expression profile closer to the pluripotent cells of primate embryos. To study chimeric competency, NHP 2CLA cells expressing constitutive GFP were injected into morula-stage rabbit and cynomolgus monkey embryos. The reconstituted embryos were cultured to the late blastocyst stage. While conventional NHP-PSCs (prior to reprogramming) returned only 20% of positive embryos harbouring less than 10 GFP^+ cells (n = 15), 90% of rabbit blastocysts analysed displayed 10 to 50 GFP^+ cells in the epiblast and trophoblast after injection of naïve-like cells. Similar results were obtained after injection of NHP-PSCs into cynomolgus monkey embryos. Thus, after reprogramming to naïve-like pluripotency, NHP-PSCs acquire competence to colonise the epiblast and trophoblast in interspecies chimaeras. Chimeric embryos are currently undergoing single-cell RNA-seq analysis to characterize the phenotype of injected NHP 2CLA cells. This work will lead to better understand the mechanisms involved in chimera generation for studying primate development.