REGENERATIVE MEDICINE

Breaking through the xenogeneic barrier

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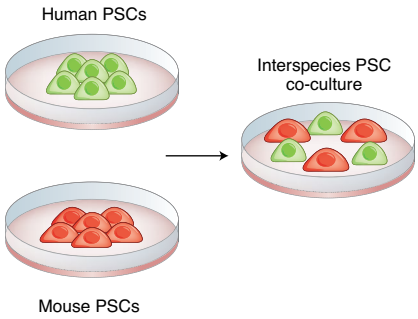
A study published in *Nature* uncovers new mechanisms by which human pluripotent stem cells (PSCs) are outcompeted in non-human embryos, which could bring us a step closer to growing human organs in animals.

The shortage of donor organs for transplantation is a major problem worldwide and several strategies are being investigated to increase organ supply, including organ production. Tissue engineering is one option for growing patient-specific organs from PSCs, but a dish might not fully reproduce the organ's natural developmental niche. Human-animal chimeras, in which human PSCs are injected into animal host embryos, is a promising in vivo alternative.

Several studies have already reported the successful generation of functional organs derived from mouse or rat PSCs in interspecies rodent models. In a 2017 pioneer study, Jun Wu and his Salk colleague Juan Carlos Izpisua Belmonte demonstrated that human PSCs can integrate into pig and cattle blastocysts. However, the resulting chimeras contained few human cells, indicating that chimera formation might be more challenging between evolutionary distant species.

In this new study, Wu, now working at University of Texas Southwestern Medical Center, and an international team of investigators developed an interspecies PSCs co-culture strategy to study the barrier to chimerism. They uncovered a mode of cell competition between PSCs of different species, which might explain the poor contribution of human PSCs to human-animal chimeras.

Cell competition was first described in *Drosophila* and refers to a process by which cells that are 'less fit' are killed by surrounding cells. Cell competition is conserved in mammals and is involved in the recognition and elimination of defective cells during development, which contributes to tissue homeostasis. "During interspecies chimera formation, xenogenic donor cells may be less fit than host cells, and thereby being targeted for elimination," explain the investigators in their report.



An in vitro strategy to study cell competition between pluripotent stem cells from different species. Credit: Marina Spence/ Springer Nature

Wu and colleagues showed that cell competition only occurs between primed PSCs from different species, and not between naïve PSCs. Stem cells cover a wide range of pluripotency. While mouse embryonic stem cells (ESCs) represent a naïve state of pluripotency, in which cells have unbiased developmental potential, mouse epiblast stem cells (mEpiSCs), human ESCs and human induced pluripotent stem cells (iPSCs) might represent a more primed state, in which cells are poised for lineage differentiation.

Using their in vitro assay, the investigators showed that primed human PSCs (H9 hESCs, H1-hESCs or HFF-hiPSCs) and mouse mEpiSCs cultured separately proliferated well, whereas human PSCs in co-culture underwent apoptosis. Mouse cells were not affected by the co-culture, allowing them to take over the human cells. In contrast to primed PSCs, naïve human PSCs were not outcompeted by naïve mouse cells in human-mouse co-cultures, demonstrating that human-mouse PSC competition is confined within primed pluripotency.

The team performed RNA sequencing on primed H9-hESCs from either co-cultures with mEpiSCs or from separate cultures to identify the mechanisms underlying human cell death. Data analysis revealed that P53 apoptotic pathway and NF-κB signaling were among the pathways overrepresented in co-culture samples. Knocking out effectors of these pathways such as P53 or P65 in human primed hiPSCs prevented the cells from being outcompeted in co-culture with mEpiSCs, confirming the role of the pathways in human cell death.

Next, the investigators injected mutant hiPSCs labeled with GFP into mouse blastocysts and performed embryo transfers to analyze human cell survival and contribution to chimera formation. At embryonic day 8-9, GFP signals could only be detected in mouse embryos injected with mutant human cells and not in embryos injected with wild-type cells, indicating that inactivation of *TP53* or *P65* had improved human cell survival and chimerism efficiency.

Further in vitro experiments demonstrated that cell competition also occurred in primate-rodent, primate-cow and rodent-cow, but not in rat-mouse and human-rhesus PSC co-cultures. "Collectively, these results extend primed PSC competition beyond human-mouse and suggest it is a more general phenomenon among different species," write the investigators.

In conclusion, the study uncovers important mechanisms of the interspecies barrier, which inhibits efficient colonization of host animal embryos with human PSCs. By identifying possible targets, these findings could facilitate the development of strategies enabling the generation of transplantable human PSC-derived organs in animals.

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