Beasts of burden: large animal chimeras using human pluripotent stem cells

Naive human pluripotent stem cells can successfully engraft into pig and cattle blastocysts, and a new CRISPR-Cas9 blastocyst complementation platform enables efficient enrichment of donor cells in targeted organs.

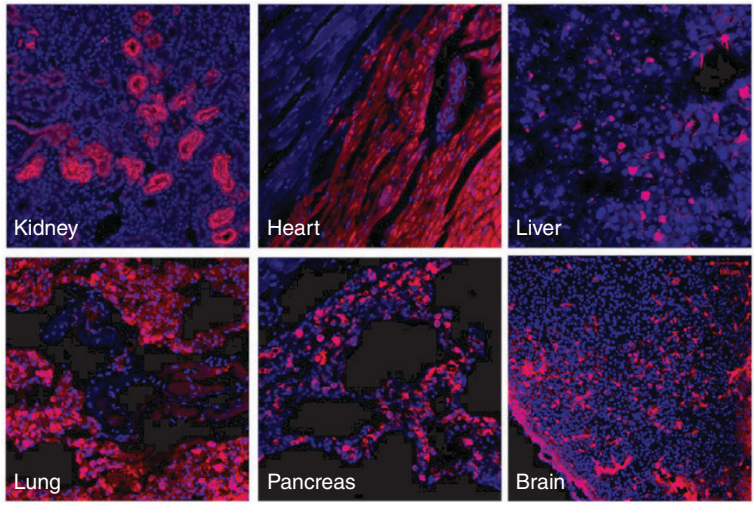
Chimeras, often depicted as Frankenstein-like creations mixing man and beast, are actually well-established and highly valuable research tools for cell and developmental biologists. Interspecies chimeras, generated from cells of two or more species, were originally designed in the 1970s to address fundamental questions in cell lineage during early embryogenesis. However, interspecies chimeras also have tremendous potential for applications in regenerative medicine, such as tissue and organ replacement.

While some efforts are underway to grow human tissues *in vitro*, Dr. Juan Carlos Izpisua Belmonte, an expert in developmental and stem cell biology at the Salk Institute, believes that growing complete human organs will also require pursuing experiments with *in vivo* systems like chimeric animals. "The idea of generating an organ, a complex three dimensional structure, in the petri dish is still very far away", says Belmonte, "…but animals already know how to do this very well, so why don't we let nature do the work for us."

However, Belmonte recognizes that the road to growing transplantable human organs in chimeric animals is also long. To get there, scientists will have to tackle multiple methodological limitations, as well as answer several basic questions in stem cell biology and cross-species embryogenesis.

In a recent paper led by Belmonte (*Cell* **168**, 473–486; 2017), he and his colleagues address two important topics in chimera research: 1) developing an efficient blastocyst complementation platform for targeted enrichment of donor cells in specific organs, and 2) directly testing how well human pluripotent stem cells (hPSCs) can contribute to chimera formation in larger, non-rodent species; the kind most likely to be used for future clinical applications.

Successful integration of donor stem cells from one species into another host species is challenging; even more challenging is trying to target those donor cells to form specific tissues or organs in resulting chimeric animals. One solution is to empty the host niche for developing particular organs or tissue types by deleting genes in the host necessary for specific cell lineages. Known as "blastocyst complementation", by removing the host's ability to develop cells for a specific organ, it unleashes pluripotent donor cells to fill the niche and generate the targeted organ.



Fluorescently labeled rat cells (red) contributing to different tissues of a rat-mouse chimera. Reproduced with permission from *Cell* **168**, 473–486 (2017).

Using the gene-editing tool CRISPR/Cas9 in rodents, Belmonte's team developed a blastocyst complementation platform that not only removes the need to rely on existing mutant mouse strains for deleting genes necessary for organ generation, but also adds the flexibility to mutate multiple genes from the same host. According to Belmonte, "Deleting one gene is not sufficient to prevent the formation of an entire organ, which is composed of many cell types from different developmental origins. So you need to use a multiplexed technology that allows you to inactivate several genes to make sure that you are preventing the formation of as many cell types as possible within the target organ."

To demonstrate the ability of their new platform to enrich donor cells in specific organs, they used CRISPR/Cas9 in mice to delete *Pdx1*, a gene necessary for mouse pancreas formation, and injected blastocysts with rat pluripotent stem cells. Resulting *Pdx1*-mutant mouse host pancreases were enriched with rat cells and were able to maintain normal serum glucose levels in the host animals.

In addition to methodological developments using rodent species, the group also expanded their evolutionary range, studying whether human induced pluripotent stem cells (hiPSCs) can integrate in larger animal species. Their results show that both naive and intermediate—but not primed—hiPSCs could integrate and survive in both pig and cattle blastocysts. However, follow-up experiments in post-implantation pig embryos found little contribution of human cells, demonstrating the need to continue looking into mechanisms separating embryogenesis between more divergent species.

While Belmonte cautions that these results won't enable human organs to be grown in other animals just yet—and probably not for many years—he does feel the developments put other important applications well within reach, like creating human-non-human chimeras for drug screening and toxicity tests, studying early human development, as well as creating animal models of human disease that have more clinical predictive value.

Dustin M. Graham