RESEARCH HIGHLIGHT

Humanizing pig kidneys via chimeric complementation

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Cell Research (2024) 34:189–190; https://doi.org/10.1038/s41422-023-00881-5

An ultimate goal in stem cell research is to use induced pluripotent stem cells (iPSCs) to generate transplantable human organs in large livestock. In a groundbreaking study by Wang et al., the researchers showcased that by combining an optimized iPSC culture, tactics to boost the survival and competitive edge of donor human cells, and a genetically vacated host organ niche, they could successfully produce a humanized mesonephros within pig fetuses, a significant leap in the field.

Recently, regenerative medicine has seen remarkable advancements, paving the way for innovative therapies and treatments for various diseases. A notably promising avenue within this discipline is the utilization of human induced pluripotent stem cells (iPSCs) to create patient-specific cells and tissues for replacement therapy, disease modeling and drug testing purposes. Human iPSCs (hiPSCs) are differentiated cells reverted to a pluripotent state, possessing the potential to generate any cell type, tissue, and organ within an adult human. Despite these exciting progresses, it remains unattainable to generate three-dimensional, functional and transplantable organs from hiPSCs in vitro. An alternative strategy involves creating hiPSC-derived organs and tissues within animals using the interspecies blastocyst complementation method.[1] However, the efficient integration of hiPSCs into host animal embryos remains an obstacle.[2,3] In a most recent study,[4] by combining several state-of-the-art technologies to improve human chimerism in animal embryos and early organs, the researchers have successfully generated a humanized mesonephros within 3–4 weeks old pig fetuses, highlighting cutting-edge advancements in stem cell technology and emphasizing the potential of bioengineering transplantable human organs within animals.

This study first tackles the challenge of integrating hiPSCs into pig embryos. Previous work from this research team highlighted the efficacy of the 4CL culture condition in stabilizing naïve human PSCs, which demonstrated enhanced interspecies chimeric potential in mouse embryos.[5] Overcoming interspecies PSC competition[6] and improving donor cell survival by blocking apoptosis[3] have been shown previously to improve human chimerism in mouse embryos. Another study by this team combined the overexpression of the pro-proliferative gene *MYCN* and the anti-apoptotic gene *BCL2* (N/B), and observed improved chimerism of conventional hiPSCs in mice, which led to the generation of human $CD34^+$ blood progenitors in *Flk-1*$^{+/-}$ mouse embryos.[2,7] Building upon this foundation, the present study combined the 4CL human PSC culture method with N/B overexpression (4CL/N/B hiPSCs) (Fig. 1a), which resulted in a marked improvement in human–pig chimerism. Another pivotal discovery in this study was the identification of a nephric-defective niche, attained by the knockout of both *SIX1* and *SALL1* genes in pig embryos, which facilitates the enrichment of 4CL/N/B hiPSCs within the pig mesonephros.

During mammalian development, the mesonephros acts as a temporary renal system, handling waste filtration and removal in the early stages before mostly retreating, allowing the adult kidney to take over. By employing 4CL/N/B hiPSCs and taking advantage of the nephric-defective niche, the researchers successfully created a humanized mesonephros in pigs (Fig. 1b). They implanted 1820 4CL/N/B hiPSCs-injected *SIX1*- and *SALL1*-deficient cloned pig embryos into surrogate sows, yielding two chimeric fetuses by embryonic day 25, and another three by day 28. Impressively, the mesonephros of these chimeric fetuses housed metanephric tubules with 40%–60% of cells being human-derived. Deep diving into this, it became evident that these human-origin cells had the capacity to differentiate into cells vital for kidney development, suggesting that 4CL/N/B hiPSCs might be used to foster the development of human kidneys in pig fetuses with kidney deficiencies. While past research has employed similar techniques to produce human tissues like skeletal muscle[8] and endothelium[9] in pig embryos, this study is the first instance where solid human-like organs have been grown within another species.

This study also examined how 4CL/N/B hiPSCs contributed to organs other than the mesonephros in the chimeric pig fetuses. Notably, these cells showed minimal participation in other organs, a discovery crucial for both safety and ethical reasons. If human cells significantly contributed to certain tissues in chimeric pigs, like the brain and germ cells, it could bring about profound ethical dilemmas, especially if these pigs matured to full term. The limited contribution of donor 4CL/N/B hiPSCs in off-target organs is reassuring from an ethical standpoint. To further alleviate such ethical concerns, a potential approach might involve knocking out genes crucial for the development of brain and germ cells in the donor hiPSCs.

While this study demonstrates promising advancements, several hurdles persist before interspecies blastocyst complementation can be considered a reliable source for transplantable human kidneys. Firstly, it is still uncertain whether the 4CL/N/B hiPSCs enriched in the pig mesonephros on embryonic days 25 and 28 can persist to the metanephros stage and function seamlessly with the host pig cells. Secondly, it is noteworthy that all attempts (including this study) to enhance human chimerism in

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Published online: 13 October 2023

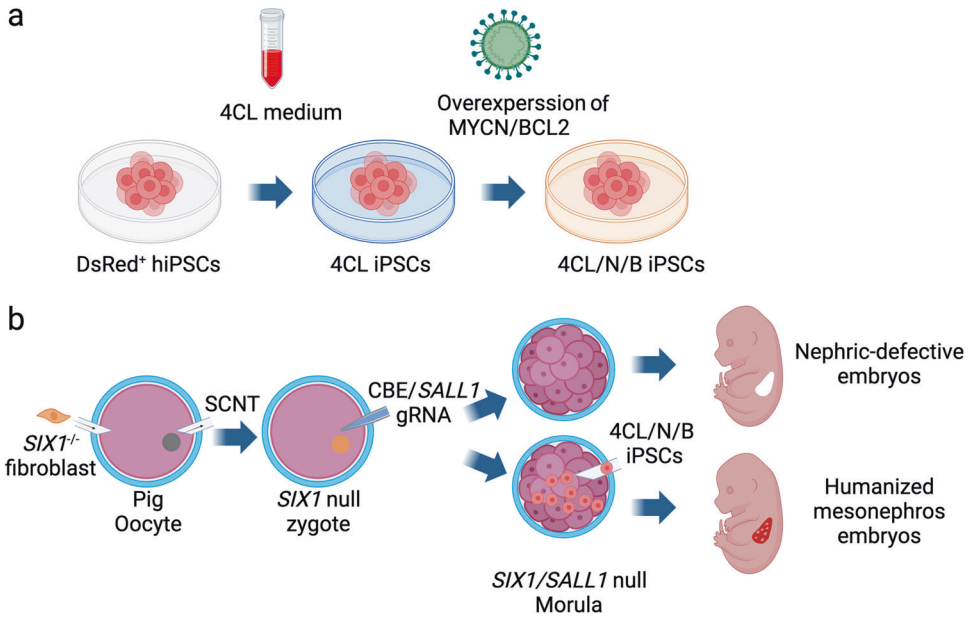

Fig. 1 Generation of humanized mesonephros in pigs. a Schematic diagram for generating human 4CL/N/B iPSCs with superior chimeric potential. **b** Schematic diagram for generating humanized mesonephros in pigs. *SIX1*-null embryos were generated by somatic cell nuclear transfer (SCNT) of *SIX1*-null pig fetal fibroblast nuclei into oocytes. *SIX1*-null one-cell embryos were then targeted with cytosine base editor (CBE) mRNA and sgRNA for *SALL1*, creating morulae deficient for both *SIX1* and *SALL1*, which were either directly transferred into surrogate sows or complemented with human 4CL/N/B iPSCs. Created with BioRender.com.

animals have leaned on genetically modifying donor hiPSCs. However, these approaches, especially as they modify largely oncogenic genes and pathways, do not seem feasible for practical regenerative medicine applications. A more desirable approach would be to boost the survival and chimerism of unaltered donor hiPSCs by tweaking the host embryos, an idea that has only begun to be explored.[10] Finally, given that the entirety of the kidney will not be composed of human cells, there is a looming concern about rejection when these chimeric organs, cultivated through xenogenesis, are transplanted into humans. Blending strategies from both interspecies blastocyst complementation and xeno-transplantation to minimize immunological barriers might pave the way for better post-transplant tolerance.

In the roughly 30 years since its first introduction, blastocyst complementation has progressed from a niche developmental biology technique to a promising avenue for addressing the worldwide organ donor shortage. The melding of hiPSCs with interspecies blastocyst complementation, leading to the creation of a humanized mesonephros in pigs, signifies a monumental step in this research trajectory. However, to truly unlock the capacity to grow human organs within animals, continued scientific innovation is essential. Concurrently, it remains vital to tackle associated ethical concerns and foster a deeper societal understanding of these developments.

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ADDITIONAL INFORMATION

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