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Interspecies chimeras

Fabian Suchy[1] and Hiromitsu Nakauchi[1,2]

By probing early embryogenesis and regeneration, interspecies chimeras provide a unique platform for discovery and clinical use. Although efficient generation of human:animal chimeric embryos remains elusive, recent advancements attempt to overcome incompatibilities in xenogeneic development and transplantation.

Addresses
[1] Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA 94305, United States
[2] Division of Stem Cell Therapy, Institute of Medical Science, University of Tokyo, Tokyo 108-0071, Japan

Corresponding author: Nakauchi, Hiromitsu (nakauchi@stanford.edu)

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Introduction

Advances in stem cell technology and tissue engineering promise clinical applications. Pluripotent stem cell (PSC) derived tissues and self-assembling organoids have been developed for use in regenerative medicine [1]. Decellularized scaffolds and 3D printers can engineer tissues with more complex, native anatomical structure [2]. Although much progress is being made, developing whole organs proves difficult [3,4]. The spatial and temporal intricacies that occur during organogenesis ensure adequate perfusion, organ size, tissue distribution and function. Since many developmental properties are not cell intrinsic, proper development requires rapidly evolving extrinsic cues that may act locally or in gradients. This environment is difficult to model *in vitro*.

Although first generated years ago, interspecies chimeras are recently gaining popularity in regenerative medicine. Defined as an organism with cells originating from at least two different species, interspecies chimeras can develop with an organ entirely composed of different species' cells. Thus, a human organ grown in a livestock animal could provide patient-matched organs and eliminate the organ transplant waiting list. This article will review recent advances in interspecies organogenesis and chimera generation, while highlighting clinical and non-clinical applications.

The organ niche

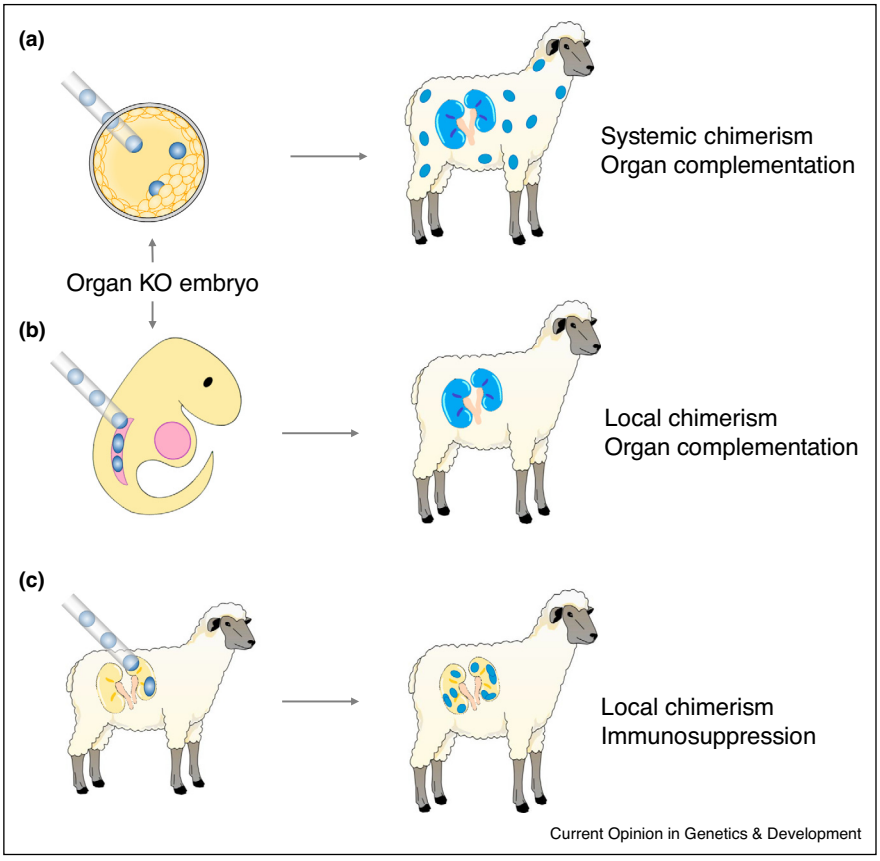
Genetic ablation of developmental pathways can disrupt organogenesis, creating an empty organ niche inside a developing embryo. When developmentally stage-matched cells from another species are delivered to the organ niche, the extrinsic cues guide development into a fully functional organ. The methods used for delivering cells to the organ niche can impact the degree of chimerism (Figure 1), requiring various practical and ethical considerations. Injecting pluripotent stem cells (PSC) into an interspecies host blastocyst can result in systemic chimerism. At the onset of organogenesis, nearby PSC-derived cells will complement the empty organ niche if donor chimerism is high enough to deliver the cells to the niche. Without competition from the host cells, many structures in the organ will develop entirely from the interspecies' cells. This method of organogenesis is 'blastocyst complementation' (Figure 1a) [5]. Another method of delivering cells to the organ niche is via direct injection to the site of organogenesis [6], resulting in higher local levels of chimerism and decreased systemic chimerism (Figure 1b). In either case, since cells are delivered prior to the onset of the immune system, they are considered as 'self' and the chimera will not reject the xenogeneic cells (self-tolerance).

In rodent embryos, ablating the niche is frequently performed by maintaining colonies of genetically modified animals [5,7–9]. Maintaining stocks of modified livestock animals would require more time and expense than in rodents. Advances in zygote gene editing via CRISPR-Cas9 has produced apancreatic pigs and sheep [10,11]. However, targeting efficiency varies by locus, and often leads to mosaic embryos. As zygote editing technology improves to produce more uniform and predictable genotypes, there will be less need to maintain large groups of genetically modified animals.

Preimplantation chimeras: blastocyst complementation

The field of interspecies organogenesis began when blastocyst complementation was used to create a mouse with a rat pancreas [7]. The reciprocal experiment was performed seven years later [12••]. In both cases, the overall pancreatic phenotype matched the host embryo, suggesting cell extrinsic cues guide organ size and morphology. Approximately 100 mouse pancreatic islets

Figure 1



Generation of interspecies chimeras and organs. Injecting interspecies PSCs into a host blastocyst may result in systemic chimerism **(a)**, while injecting progenitor cells at later developmental stages can limit systemic chimerism **(b,c)**. Donor cell rejection must be considered if injecting cells after the onset of immune development (c). If development of a specific organ is disabled (KO) prior to the onset of organogenesis, engrafted donor cells can fill the empty organ niche to develop a functional interspecies organ (a, b).

generated in a rat were transplanted into a diabetic mouse. Due to a small number of contaminating rat cells, immunosuppression was used for five days. The mouse maintained normal blood glucose levels for over year and was cured of diabetes [12••]. This proves that tissues generated *in vivo* through interspecies blastocyst complementation are functional after transplantation and can be utilized to treat diseases.

At least two states of pluripotency have been characterized [13]. PSCs in the 'naïve' and 'primed' state only engraft when injected into the preimplantation embryo or post-implantation epiblast respectively [14,15•]. Thus, resetting human PSCs to a naïve-like state may be necessary to enable robust blastocyst engraftment. Many human naïve-like cells have been reported [16]. The Jaenisch group injected ~3000 mouse preimplantation embryos with naïve human PSCs cultured in five different conditions [17••]. When analyzed a few days after gastrulation, less than 1% of the implanted embryos were chimeric, all resulting from only two of the five culture conditions. More recent culture systems have produced cells with totipotent-like characteristics. Human cells cultured in these conditions seem to form human:mouse chimeric embryos more frequently (~20%) and with a higher proportion of human cells [18,19]. Collectively, this highlights the importance of cell type and developmental stage when creating interspecies chimeras.

Similar experiments in which human PSCs were injected into pig embryos resulted in low levels of chimerism when measured a few weeks after implantation [20••]. Again, cell culture condition influenced the frequency of chimerism. Human:pig chimerism was predicted to be around 1 human cell in 100 000 pig cells. It is unknown if ablation of an organ niche can be complemented in cases of extremely low chimerism, as a minimum number of progenitors may be necessary to develop into an organ [21]. Interspecies blastocyst complementation has not yet been achieved outside of rodent species.

Efficiency and the interspecies barrier

Rats and mice diverged approximately 20 million years ago [22]. Under controlled conditions, formation of