Humanized organs in gene-edited animals

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Treatment of chronic diseases has resulted in the successful use of cell therapy for the treatment of hematopoietic diseases and cancers as well as device therapies for the treatment of heart disease, diabetes and osteoarthritis. These therapies, while effective, have not been broadly applied to end-stage disease. Currently, curative therapies for advanced end-stage organ failure require transplantation, which is limited by donor organ availability. While millions of patients could benefit from such therapy, the scarcity of organs severely limits the number of transplantations that are performed. For example, it is estimated that more than 100,000 Americans would benefit from a heart transplant yet approximately 2200 adults receive a heart in the USA annually [1]. Furthermore, transplantation of organs requires lifelong immunosuppression, which is associated with significant morbidity [2]. This disparity has fueled intense interest focused on alternative organ sourcing and regenerative medicine.

Groundbreaking scientific advances are bringing the scientific field closer to the reality of developing human organs in nonhuman animals. First, the advances in developmental biology have identified master regulators that are both necessary and sufficient to specify stem cells and direct them to differentiate to distinct lineages [3]. Second, the ability to reprogram human somatic cells to a pluripotent stem cell state, human induced pluripotent stem cells (hiPSCs), has revolu-

tionized the field of regenerative science and medicine [4,5]. Third, genome-editing technologies, such as clustered regularly interspaced short palindromic repeat, allow for site-specific genome editing [6]. Fourth, the ability to successfully perform somatic cell nuclear-transfer technology (i.e., cloning) in large animals has allowed for the genetic engineering of large animal models. The intersection and combination of these four emerging technologies makes feasible the ability to delete the genes that govern tissue or organ development in a host, thereby establishing a niche for humanized cells. In addition, the use of complementation experiments, where hiPSCs are transferred to a mutant blastocyst, followed by the transfer into a pseudo-pregnant host, could result in the potential rescue of the host phenotype rescue with a humanized organ. Therefore, it may be possible to engineer personalized organs in large animals and/or engineer unique human disease models in a large animal for preclinical testing of potential therapeutic agents, which is the focus of this editorial.

Human:animal chimeras

The use of human cells or lineages in a non-human animal has been extensively pursued in biomedical research. For example, the incorporation of human hematopoietic stem cells into early, preimmune fetal lamb embryos was demonstrated in the 1990s [7]. These investigators observed significant,

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long-term, multilineage engraftment of these cells in sheep bone marrow and blood. Additionally, in 2005, functional human neurons in the mouse were developed by injecting human embryonic stem cells into the ventricles of mice [8,9]. Humanized liver models in mouse have been well established and are currently used for the study of pharmacokinetics and toxicity. In 2001, the repopulation of a mouse liver with human hepatocytes was described [10,11]. In 2004, human hepatocytes were transplanted into an immunodeficient mouse model to generate chimeric mice with an 80–90% humanized liver [12]. The utility of these chimeric mice in studying human toxicity and dosing and disease is well recognized [13]. More recently, 3D vascularized and functional human livers have been generated by transplanting human liver buds, developed *in vitro*, into mice [14]. An additional example of human:animal chimera is the use of teratoma formation in immunodeficient mice to assess the pluripotent potential of human stem cell populations. In this fashion, this teratoma production has emerged as a gold standard to characterize the pluripotent capacity of human stem cells as they have been shown to differentiate to lineages of all three germ layers (i.e., ectoderm, endoderm and mesoderm derivatives). Collectively, these examples highlight the extensive use of human:animal chimerism as a tool in the research laboratory.

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Various studies have demonstrated the capacity for targeted organ chimeras using blastocyst-complementation strategies. For example, a rat pancreas was produced in a mouse by the process of blastocyst complementation [15]. In these studies, blastocysts mutant for *Pdx1*, the master regulatory gene for pancreatic development, were injected with pluripotent stem cells from wildtype rats. Transfer of the pluripotent stem cells from wildtype rats injected blastocysts and, subsequently, into surrogate mouse dams gave rise to mouse chimeras with functional pancreata composed of rat cells. These studies emphasized the importance of generating blastocysts, deficient for a key developmental regulatory factor, in which the embryo completely lacks the target organ. The blastocyst-complementation strategy has also produced organs such as the kidney and liver in rodents, and recently, the pancreas in pigs [15–17]. The results of this latter study are significant, because it supports the notion of generating human patient-specific organs in pigs that can be subsequently used for transplantation or advanced therapies.

Ethical issues confronting human:animal chimera research

The ethical issues surrounding the generation of human:animal chimeras (otherwise known as humanized models) are multifaceted. Some concerns involve sensitivities with regard to human qualities existing within an animal [18]. The adage, “what we don’t know won’t hurt us” might be applied were we to consider a large animal with a human pancreas, for example, versus a large animal with human ears or human hair or human skin. The sight of a creature that is obviously part man:part beast is likely to be offensive to some, if not to many. On the other hand, a large animal that can serve as a factory for tissues or organs to cure disease may be much more desirable and compatible with the current societal uses of livestock. Major ethical issues that are confronting this human:animal chimeric field include: the contribution of human stem cells to the brain (and the concern that the chimeric animal may have human thought); the contribution of human stem cells to the germline (the need to prohibit any reproduction of the chimeric animal); the need to limit the contribution of human stem cells to the chimera (what would be an acceptable human contribution to the chimera – would 10% be acceptable – would one germ layer or less be acceptable?); should these initial chimeric studies be limited to the early developmental stages (i.e., first trimester)?; should patient consent forms specify whether their cells can be used for these human:animal chimeric analyses?; what does it mean to be human – at what stage does a chimera become human?

While no international policies exist governing the creation of human:animal chimeras, Canada passed the Assisted Human Reproduction Act in 2004. This law expressly forbids the ability to “create a chimera, or transplant a chimera into either a human being or a non-human life form” (Assisted Human Reproduction Act [Section 8 Consent] Regulations 2007). In September, 2015, this issue was escalated in the USA when the NIH placed a moratorium on the funding of studies that proposed the generation of human animal chimeras (NIH Notice Number NOT-OD-15-158). A panel was convened by the NIH in November 2015 to formally consider these human:animal chimeric studies and begin a dialogue regarding the impact and safeguards required for these studies. In response to the NIH moratorium, scientists have urged the NIH to reinstate the funding of this work, as they estimate that the ban threatens to halt the progress of these emerging technologies and their impact on regenerative medicine field [19].

Challenges for the field

Additional scientific challenges have emerged for this field of study. Currently, the efficiency regarding

blastocyst complementation of human:animal cells is limited. Further studies will be needed to define the ground or naive state of human stem cell populations (hiPSCs). In contrast to the primed state of hiPSCs, the naive state is characterized by a distinct molecular signature, a hypomethylated genome and a higher cloning efficiency. In addition, the immunological status of the gene-edited animal may need to be modified to prevent rejection of the human lineage in the chimera. Furthermore, the human stem cells may need to be genetically modified to limit their contribution to the developing animal brain or germline. These, and other scientific challenges will need to be addressed in the near future.

Conclusion

In summary, technological advances in numerous fields allow for the advent of novel humanized models for the

study of disease and for the generation of organs for transplantation. It will be important to thoroughly, yet rapidly, address any ethical concerns that exist regarding these initiatives. These studies are now feasible and hold extraordinary potential for understanding and treating deadly diseases and may one day serve as an alternative source of transplantable organs.

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