Human-Animal Chimeras in Biomedical Research

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Chimeras are individuals with tissues derived from more than one zygote. Interspecific chimeras have tissues derived from different species. The biological consequences of human-animal chimeras have become an issue of ethical debate. Ironically, human-animal chimeras with human blood, neurons, germ cells, and other tissues have been generated for decades. This has facilitated human biological studies and therapeutic strategies for disease.

Introduction
A chimera is an individual composed of somatic and, in certain cases, germ line tissues derived from more than one zygote. There are different ways to generate tissue chimerism, including mixing embryonic cells from two individuals, transplanting fetal or adult tissues from one individual into another individual, or grafting embryonic stem (ES) cells or their differentiated products into another individual. For example, two genetically distinct 8 cell mouse preimplantation embryos whose zona pellucidae have been removed can be pushed together in a tissue culture dish and then grown in vitro to form a single blastocyst. Transfer of this blastocyst into the uterus of a foster mother often results in a mouse with somatic and germ cells of both genotypes, a so-called aggregation chimera. Tissue chimerism can also result from clinical treatments of disease. A patient who receives a successful tissue or organ transplant (e.g., bone marrow) would likely have the adult donor tissues obtained from a genetically distinct individual, i.e., such a patient would be a bone marrow chimera. If the donor tissue and recipient are of different species, then an interspecific or cross-species chimera is generated. Human-animal interspecific chimeras have been created by grafting human cells and tissues into the embryos, fetuses, or adults of vertebrate model organisms. The derivation of human embryonic stem (hES) cells has created the opportunity to use these pluripotent cells to generate human-animal chimeras that has led to recent ethical discussions (McLaren, 2007). These ethical issues are discussed in the accompanying articles by Hyun et al. (2007) and Lensch et al. (2007).

Laboratory animals are routinely used to model human biology and disease but are not human and therefore cannot fully replicate human physiology. Thus, the primary goal of human-animal chimera research is to produce human cellular characters in animals. The animal carrying the human tissue can then be examined or treated to investigate human-specific biological processes and disease without experimentation on human individuals. The general public and most scientists may not realize that human-animal chimeras have been routinely produced for decades and are generated daily in biomedical research laboratories throughout the world. Here we review different types of human-animal chimeras that have been used in biomedical research. We focus our review on human-animal chimeras generated by transplanting human tissues into vertebrates, predominantly rodent models. These different types of human-animal chimeras have provided important insights into fundamental biological mechanisms and the development of therapeutic protocols for human disease.

Xenografts into Immune-Deficient Animals
Human-animal chimeras are typically generated by grafting human cells or tissues orthotopically or heterotopically into an immune-deficient animal. The host may be a fetus whose immune system is immature and has yet to establish “self” or a postnatal animal with a genetic mutation that causes the loss of specific immune system components. Engraftment of human cells into wild-type postnatal animals whose immune systems have been established can also be accomplished by immunosuppression with drugs (e.g., cyclosporin).

Classically, the immune-deficient athymic nude mouse has been used for decades as a recipient for human tissue grafts to generate human-mouse chimeras. Nude (Foxn1-nu) is a recessive mutation that results in, among other phenotypes, loss of T cells. This T cell deficiency in the nude mouse is sufficient to allow many types of xenograft tissues to survive and grow, including different types of normal fetal and adult human tissues (Povlsen et al., 1974). Abnormal or diseased human tissues can also be transplanted into animals. The successful growth of human tumors in animals was pioneered by transplantation into immune-privileged sites (e.g., the rabbit or guinea pig anterior chamber of the eye or Syrian hamster cheek pouch). The advent of the nude mouse greatly facilitated these types of studies, and indeed, this in vivo assay is one of the fundamental experimental paradigms for cancer research (Rygaard and Povlsen, 1969). Pieces of human tumors are grafted under the skin of nude mice, providing a bioincubator for tumor growth. The mouse essentially becomes a cancer patient whose tumor can then be manipulated in various ways to understand cancer...
mechanisms and to test therapeutic protocols for human cancer.

Although nude mice are sufficient for many types of xenograft experiments, their immune systems still have B cells and natural killer (NK) cells and are able to fix complement, and thus are capable of hindering the engraftment of some types of human tissues, notably hematopoietic cells. Mutations in Prkdc (scid, severe combined immunodeficiency), Lyst (beige), Btk (xid, X-linked immunodeficient), B2m (β2-microglobulin), Prf1 (perforin), Ilr2γ (IL-2 receptor γ), and Rag1 and Rag2 mice have also been used singly or in various combinations to further inactivate other components of the host immune system (Shultz et al., 2007). In addition, genetic background can also influence host immune function. Mice on the nonobese diabetic (NOD) inbred genetic background combined with the Prkdc<sup>scid</sup> mutation (NOD-SCID) show greater human hematopoietic cell engraftment than classical SCID mice. More recently, NOD-SCID with Ilr2γ and some of the above mutations have shown the greatest human hematopoietic cell transplant contributions. Human-mouse chimeras with tissues engrafted at orthotopic sites have been termed “humanized.” The utility of human-mouse hematopoietic chimeras has been recently reviewed (Shultz et al., 2007).

Another general approach to generate human-animal chimeras is to add other mutations to or chemically treat or irradiate immune-compromised (e.g., nude, SCID, etc.) animals to make them also deficient for nonimmune system tissues. The deficient or absent tissue opens a niche for the engrafted human nonimmune system cells. For example, sublethal irradiation of NOD-SCID mice is necessary to “knock down” host hematopoiesis for engraftment of human hematopoietic stem cells. Using this general approach, human tissues can contribute significantly or completely to a specific tissue or organ in the animal. Many of the grafted human tissues have significant regenerative potential and long-term colonizing ability (i.e., stem cells), whereas other tissues can only terminally differentiate (e.g., ovarian primordial follicles). These human-mouse chimera models have been useful for studying human hepatitis virus infections and human liver-specific metabolic responses to drugs.

**Animals with Human Neurons**

Among the most ethically charged forms of human-animal chimerism are those that incorporate human neurons into the host central nervous system (CNS). Human-animal neural chimeras have been generated in two fundamental ways: (1) human fetal brain cells are used directly, cultured as neurospheres to isolate neural stem/progenitor cells, or sorted using cell surface markers and transplanted into embryonic or newborn animals; (2) hES cells are differentiated into neural precursor cells or differentiated neurons in vitro and then transplanted into embryonic, newborn, or adult animals. The site of transplantation is typically a brain ventricle, providing the graft access to large areas of the CNS, but these cells can also be transplanted directly into host neural tissue.

Extensive neural and glial brain chimerism was documented using human fetal brain tissue transplanted directly, as a single cell suspension, or first cultured in vitro as a monolayer or as neurospheres, into the cerebral brain vesicles of wild-type fetal rats (Brüstle et al., 1998). Human fetal brain tissue has also been dissociated and then sorted for human central nervous system stem cell (hCNS-SC) activity, using antibodies that enrich for hematopoietic stem cells (Uchida et al., 2000). These hCNS-SCs were subsequently cultured to generate neurospheres, then dissociated and injected into the lateral ventricles of newborn NOD-SCID mouse brains. Differentiated human neurons were found throughout the brains of these human-mouse neural chimeras up to 7 months posttransplant. To investigate the ability of human fetal brain cells to engraft a primate brain, human-monkey chimeras have been generated by transplantation of human fetal neural stem cells into the lateral brain ventricles of fetal monkeys (Ourednik et al., 2001). Analysis of the host brains 1 month posttransplantation revealed the presence of human neurons and glia and undifferentiated donor cells.

hES cells have also been a source of neural progenitors and differentiated neurons to test their ability to incorporate into a developing or mature animal CNS. hES cells were cultured to generate neural cells and transplanted into the cerebral ventricles of wild-type newborn mouse brain immune suppressed with cyclosporin (Zhang et al., 2001). The grafted human cells were found in multiple regions of the host brain and had differentiated into neurons and glia. Like mouse ES cells, hES cells can now be directed in vitro to specific neuronal fates. Recently, hES cell-derived motor neurons generated in vitro were transplanted into the spinal cords of chick embryos and adult rat spinal cord (Lee et al., 2007). The human motor neurons were able to quickly incorporate into the spinal cord of the chick embryo, retain motor neuron markers, and extend axons from the spinal cord to peripheral tissues (i.e., muscles). The human motor neuron grafts into the adult rat spinal cords were able to survive for up to...
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and functional spermatogenesis maturation arrested by
drug therapy directly into the seminiferous tubules of
nude mice whose endogenous spermatogenesis had
been ablated by the drug busulfan. They found that the
mouse tubules were successfully colonized by the human
spermatogonial stem cells that proliferated and persisted
for up to 6 months posttransplantation. However, the hu-
man spermatogonial stem cells did not progress to meio-
sis. Subcutaneous grafting of testicular tissue fragments
in immune-deficient mice has successfully yielded mature
and functional spermatooza from diverse mammalian
species (Orwig and Schlatt, 2005). Subcutaneous trans-
plantation of human prepubertal and adult testicular tissue
fragments into immune-deficient mice has also been at-
ttempted, showing that human spermatogenesis can survive
in mice (Geens et al., 2006). While success with human
testicular tissue has been limited, the subcutaneous trans-
plantation assay has been used to generate fertilization-
competent spermatooza from prepubertal rhesus monkey
testicular fragments (Honaramooz et al., 2004). Thus, with
more research it may be possible to incorporate this tool
with strategies to preserve the germline of young boys
undergoing chemo- and radiotherapies that cause
sterilization.

The study of human female germline development and
its preservation has also been pursued using human-
mouse chimeras. Ovary transplantation in the mouse is
a classic method for preserving the germline of female
mutants that may have reduced fertility because of comor-
bidty. Adult or even fetal mouse ovaries are transplanted
orthotopically into histocompatible wild-type hosts, re-

sulting in the generation of oocytes that can be fertilized
by normal matings to produce progeny. The ovaries
from many different species of mammals have been trans-
planted into immune-deficient mice, including marsupials,
cow, cat, elephant, and human, yielding antral follicles
(Paris et al., 2004). The utility of the xenograft assay for
preserving the female germline of a mammal was estab-
lished by the production of live mouse young from mouse
ovaries grafted into nude rats (Snow et al., 2002). The cor-
tex of the human ovary contains hundreds of primordial
follicles, and small fragments are amenable for cryopres-
ervation. Frozen and thawed human ovarian fragments
have been transplanted subcutaneously or under the renal
capsule of nude or SCID mice, yielding antral follicles. This
has allowed the effects of various hormone manipulations
on follicle development to be explored. In one study, cry-
opreserved ovarian fragments from 18 human lymphoma
patients were grafted into SCID mice. None of the recipi-
ent mice developed lymphoma, whereas mice receiving
lymph node tissue from one of the lymphoma patients de-
veloped B cell lymphomas (Kim et al., 2001). This study
suggests that ovarian tissue fragments isolated from
patients prior to treatment for lymphoma could be pre-
screened in human-mouse chimeras prior to autotrans-
plantation to restore fertility.

Mice with Human Germ Cells

One recurring concern that has been raised about hES
cell-mouse chimeras is that the animals could potentially
generate human germ cells (McLaren, 2007). Although
hES cell-mouse chimeras that have progressed to the
stage of germ cell differentiation have yet to be generated
(James et al., 2006), other types of mice carrying human
germ cells have already been produced. These types of
experiments are designed to investigate the mechanisms
that regulate human germ cell development and to de-
velop ways to preserve the germline of patients (e.g., a pa-
tient undergoing chemotherapy or radiation therapy for
cancer). Initial experiments described the transplantation
of 14–22 gestational week human fetal testis fragments
subcutaneously into nude mice, resulting in the persist-
ence of human gonocytes at 23–57 days posttransplanta-
tion (Povlsen et al., 1974). Nagano et al. (2002) trans-
planted testicular cells from men with obstructive
azoospermia or spermatogenesis maturation arrested by
drug therapy directly into the seminiferous tubules of
nude mice whose endogenous spermatogenesis had
been ablated by the drug busulfan. They found that the
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Human ES Cells Transplanted into Animals

One of the pivotal biological discoveries in recent years
has been the derivation of pluripotent hES cells. hES cell
pluripotency has been screened by in vitro differentiation
assays. In vivo, the predominant assay for pluripotency
has been teratoma formation. Teratoma formation from
hES cells injected subcutaneously into immune-deficient
mice results in a tumor with many, potentially all, differen-
tiated body cell types. Human-mouse chimeras carrying
hES cell-derived teratomas are discussed in depth in
Lensch et al. (2007).

There are only a few reports directly combining hES
cells with the embryos of vertebrate model organisms. Chick embryos were used as a host to test hES cell po-
tency (Goldstein et al., 2002). Clumps of 100–200 hES
cells were grafted into somite stage embryos and incu-
bated for 1–5 days to create human-chick chimeras. The
transplanted hES cells were able to survive, proliferate,
and differentiate into epithelia, dorsal root ganglia, and
neural rosettes with differentiated human neurons associ-
ated with the host neural tube. There is currently one re-
port describing the results of injection of hES cells into
the cavities of mouse blastocysts (James et al., 2006). The
injected hES cells were incorporated into the inner
cell mass of the blastocysts and continued to express
the pluripotency protein, Oct3/4. Of 28 hES cell-mouse
chimeric blastocysts transferred into the uterus of female
foster mice and collected 5 days later at head-fold stages,
only four embryos had hES cell-derived cells and only one
of the four appeared normal, containing just ten hES cell-
derived cells in foregut endoderm and neuroepithelium. It
is currently unclear why there was such poor incorporation
of the hES cells into the mouse embryo. Finally, hES cells
have been injected directly into the brain ventricles of fetal mice (Muotri et al., 2005). The hES cells were marked by enhanced green fluorescent protein (EGFP) expression and ~10^6 were injected through the exposed uterus into the lateral ventricles of 14 days postcoitus mouse fetuses and then returned to the mothers to complete gestation. Differentiated and functional human neurons and glia formed that were incorporated into the brains of the host animal. Interestingly, the size and timing of differentiation of the human cells appeared to be regulated by the host environment. In addition, no hES cell-derivered teratomas formed in this human-mouse chimera model. Although the above examples suggest that hES cells may have remarkable regenerative potential in embryos and fetuses, there are currently too few examples of these types of experiments to make many conclusions.

**Human-Animal Chimeras: An Established In Vivo Paradigm for Biomedical Research**

Model organisms offer in vivo systems to study fundamental biological processes, providing insights into human physiology. However, these animals are not human and have limitations for studying specific human cellular characteristics. Practical and ethical concerns preclude direct studies on humans. Thus, human-animal chimeras provide an in vivo system for studying human tissues without experimentation on human individuals. Most of the biological outcomes from the human-animal chimera studies presented above are no different than what might occur if hES cells were mixed with animal embryos. As with all animal experimentation, there must be regulatory oversight (see Hyun et al., 2007). However, current discussions about the potential biological outcomes of hES cell-animal chimeras should consider the long heritage of human-animal chimera research that has provided important insights into human physiology, disease, and drug discovery.

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