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Bracha Sklar
Touro College

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Human Animal Chimeras For Therapeutic Protocols

By Bracha Sklar

Bracha Sklar will graduate in January 2016 with a BS degree in Biology.

Abstract

Research on humans is limited, therefore human animal chimeras have been used to study human systems. A Human animal chimera is an animal containing animal and human cell lines. The primary goal of human animal chimera research is to establish an animal with human cellular characters, which can and should more realistically be able to imitate as closely as possible the in vivo situations in humans. This research is very important, because it allows scientists to study human systems in vivo using a humanized animal model. However ethical issues arise when experimenting with humans and animals being mixed together. Using information found on Touro College's database, this article reviews studies done using human animal chimeras, and their potential benefits for therapeutic protocols.

Introduction

A primary goal in biological research is to understand human development, however research on humans is restricted. Although isolation of human embryonic stem cells (hESCs) and the generation of human induced pluripotent stem cells, have led to more options in the field of human biology, there is still need to analyze these cells in an in vivo setting. As a result, many researchers are now turning to human animal chimera research (Hermerén, 2015). The definition of a chimera, suggested by Lensch et al. (2007), is an organism containing cells from two or more individuals of the same or different species. Behringer (2007) proposed, that a chimera is an organism with two cell lines, where the individual is "composed of somatic and, in certain cases, germline tissues derived from more than one zygote."

There are different types of chimeras, Tetragametic chimeras, arise in many cases from in vitro fertilization (IVF), a route of fertilization, in which an egg and sperm are combined in a laboratory dish, and the resulting embryo is then transferred into the uterus. Because the ensuing embryos were grown in close contact, Tetragametic chimeras can result (Granzien, R.R, 2014). Tetragametic chimeras also occur when twin zygotes fuse together and develop into one body. As a result, the individual's tissues are derived from more than one zygote, and contain two sets of DNA. There have been cases where this happened, and affected the individual later on in life, when she has her own child and if a DNA test is done, it might not match up. It can be her 'sisters' genes that she fused with that matches her children's. Further testing will prove it's her child by matching the same genotype in some parts of her body. A person who has two different eye colors, can be from the fusion of two zygotes, among other causes. Consequently, someone born with male parts and female parts can be male and female early embryos that merged together (Norton and Zehner, 2008). But here, chimeras spoken about are human animal chimeras, which are being studied more by scientists in the hope to discover cures for diseases, such as Alzheimer's and Parkinson's and to develop vaccines, for example to help fight HIV.

Human animal chimeras have been created by "grafting human cells and tissues into the embryos, fetuses, or adults of vertebrate model organisms (Behringer, 2007)." Currently researchers and

scientists are experimenting how the use of chimeras can advance the field of medicine. Scientists are utilizing the unique ability of stem cells to proliferate and differentiate into various cells. They are using the idea of stem cell research, but with a twist. Scientists are injecting human stem cells (hCSs) into animal embryos for the purpose of studying human diseases. When a fetus is forming, the immune system is not mature enough to attack anything foreign, therefore modifications can be made in an embryo. For example, early discovery of an abnormality can be treated with stem cell administration. The injected cells can freely proliferate and/or differentiate into new cells, replace the diseased ones, and regenerate damaged tissue. Although this concept might sound relatively new, the idea of chimeras are already being practiced today, by using pig heart valves for humans and using animals for skin grafts (Cooper, 2012).

The study of biological systems in humans is largely regulated to in vitro models that lack the mechanisms and intricacy of living organisms. The complexity of a biologic structure can only be precisely and realistically replicated by an in vivo system. In vivo studies in laboratory animals are routinely used to model human biology and disease but they are not human and therefore cannot fully replicate human functioning. Transplanting human cells into animals provides a way to study human systems in vivo (Sun et al., 2007). Humanizing an animal, by implanting human blood, neurons, germ cells and other tissues into it, results in a chimera. Human animal chimeras, containing animal and human cell lines have been generated for decades to facilitate human biological studies and therapeutic strategies for disease (Behringer, 2007).

This paper reviews studies done using human animal chimeras. Although research with chimeras shows great potential for insight into diseases and medicine, it also raises unique ethical issues that must be considered. Are the benefits of using human animal chimeras for therapeutic protocols worth the possible risks?

Materials and Methods

In order to answer the question proposed above, many research papers and journal articles with relation to this area have been read. Touro College's library data base (tourolib.org) was utilized to search for relevant studies and reviews, with

databases such as ProQuest, and EBSCO. Further research was done by looking up references cited from review articles to find original papers. All research accumulated was used in an attempt to determine if the benefits of humanized animals outweigh the possible risks.

Discussion

There have been a number of studies using human animal chimeric mice as an *in vivo* system to study how stem cells proliferate, to test diseases, and to study in more detail the biologic systems. Often mice are used, however fetal sheep and fetal goat, during the pre-immune stage of development, have also been used as a surrogate animal model for human stem cells. The pre-immune stage allows for the transplant of human stem cells, and the resulting human animal chimera serves as a unique and clinically relevant xenograft animal model for assessing the differentiation potential of hSCs *in vivo*. However from a scientific perspective, large animals are not a suitable model for mechanistic research and the experiments reviewed here utilize mice, as a host for human cells. Small animal models, such as mice and rats according to scientists are ideal models to use. The advantages of using small animals is that they contain naturally occurring migration patterns of stem cells, and provide the availability of extending homing and engraftment sites. Additionally, the presence of tissue and organ specific signals from niche, greatly facilitate the widespread distribution of human donor cells. It is these reasons that compelled scientists to develop an influential small animal model, also known as a humanized animal for pursuing hSC basic research (Sun et al., 2007).

Stem Cells

In one study, a human/rat xenograft animal model was generated by transplanting human low-density mononuclear cells (hMNC's) from human umbilical cord blood (hUCB) into fetal rats to study how the human donor stem cells behave *in vivo*. Numerous methods including flow cytometry, Polymerase Chain Reaction (PCR), and immunohistochemistry (IHC) assay were used to test the human donor contribution. Flow cytometry detected that out of 29 recipients, 19 had human leukocyte common antigen, CD45+ cells in peripheral blood (PB). PCR analysis on 11 different adult tissues showed that 14 out of 19 CD45+ animals possessed donor derived human engraftment in multiple tissues for example, the liver, spleen and thymus. The differentiation of human donor cells in the liver was assessed by IHC with the use of human specific antibody against the hepatocyte marker; CK18. The recipient liver, and the human liver had cells that expressed human CK18, while normal control rats did not. IHC examination revealed that in the chimeric adult spleen of recipients, many donor derived human cell populations expressed CD45 marker indicating the human spleen-specific differentiation of donor derived human

cells. Differentiation of hMNC's was evaluated in the rat thymus using IHC with a human specific antibody against CD45. At 3 weeks, 2 months and 6 months after *in utero* transplantation, multiple human cells recognized in the recipient thymus strongly expressed CD45. Human umbilical cord derived cells expressing CK18 underwent site specific differentiation into CK18+ human cells in the recipient liver and CD45+ human cells in the recipient spleen, which demonstrates that after *in utero* transplantation of hMNC's, they successfully engraft into the liver and spleen of recipients. "Subsequently, the long term that survive in these organs are then actively influenced by niche signals to contribute in organogenesis (for example liver and spleen) recipients in the xenogeneic competitive settings." This experiment concluded that a human rat chimera was successfully developed in which xenogeneic human cells exist for up to 6 months. This provides a great *in vivo* model to study how stem cells work (Sun et al, 2007).

In another study, hESCs were implanted into the brain ventricles of embryonic mice, and found to differentiate into functional neural lineages, generating mature active human neurons that successfully integrate into the adult mouse forebrain. This study reveals insights to recognition of common signals for neural differentiation throughout mammalian evolution. The results showed that hESCs, when transplanted into the ventricle of the developing mammalian brain, can give rise to neuronal and glial lineages, suggesting that they are "responsive to environmental cues that regulate cell fate determination and differential migration." This human animal chimera model provides an *in vivo* approach to study human neural development. An approach which can be used to study human neurodegenerative and psychiatric diseases, and a prospective technique to speed up the screening process for therapeutic drugs in the future (Muotri et al., 2005).

Disease Model

In another experiment, an animal with a humanized immune system was used to serve as a human disease model for Human Immunodeficiency Virus type 1 infection (HIV-1). Rag2^{-/-}vc^{-/-} mice, when neonatally injected with human CD34+, develop a functional human immune system, with human hematopoietic cells found in the thymuses, PB, spleen, and bone marrow (BM) of the animals. Rag2^{-/-}vc^{-/-} mice become infected with HIV-1 when injected with CCR5 tropic HIV-1. HIV-1 infection is characterized by constant virus replication and a gradual loss of CD4+ T cells and T-cell function. After being injected with CCR5 tropic HIV-1, there was a productive infection of human cells in PB, thymus, spleen tissue, and BM, and ratios of CD4 (+) T cells to CD8 (+) T cells declined. Infection of the mice with 5000 TCID₅₀ of R5 HIV-1 resulted in a productive infection in 3/3 animals. HIV replication was detected in PB, thymuses and spleen and BM was also positive for HIV-1. When a 10 fold

smaller amount 500 TCID₅₀ was used for infection, only 2/5 were found to be productively infected with HIV-1 in PB and all lymphoid organs. This study demonstrated that HIV-1 can be detected in multiple lymphoid tissues of Rag2^{-/-}vc^{-/-} mice, using low doses of CCR5-tropic HIV-1. This mouse, consisting of a human hematopoietic system, can serve as a small animal model for investigating HIV-1 pathogenesis and testing potential HIV-1 therapies (An et al., 2007).

In the next study, a chimeric mouse, that sufficiently mimics the pathophysiological micro-environment in human liver, was established as a unique experimental model to study human liver cancer metastasis (Fujiwara et al., 2012). This was discovered by expression of an albumin-urokinase plasminogen activator (Alb-uPA) transgene in the mouse that is hepatotoxic, resulting in the progressive destruction of the mouse liver. It was found that when primary healthy normal human hepatocytes were transplanted into this mouse, the human hepatocytes were capable of reconstituting the host liver, highlighting the generous capacity of hepatocytes to regenerate. This liver reconstitution mouse model has led to a mouse with a humanized liver, by the development of mice with livers that are reconstituted by engrafted human hepatocytes. This model can be useful for in vivo testing of anti-cancer drugs and for studying the mechanisms of human cancers (Behringer, 2007). Based on this, scientists theorized that the chimeric mouse can be used as an animal model to investigate the underlying mechanisms of tumor metastasis into the liver, where the “parenchyma is composed largely of normal and healthy human hepatocytes (Fujiwara et al., 2012).”

Human Organs

Human animal chimeras can be used to create complete human organs by growing organs made exclusively from human cells in a chimeric animal, such as a pig, that could potentially be used for organ transplants (Hermerén, 2015). It was shown, that when a mouse that is deficient in T and B cells was injected with mouse embryonic stem cells, the T and B cells generated were the donor derived mouse embryonic stem cells. The donor stem cells compensated for the lack of T cells by producing T cells. It was hypothesized that the same may be true for organs. That if an animal that is deficient in an organ is injected with stem cells, the stem cells might compensate for what its lacking and grow into that organ.

A study was done where mouse wild-type pluripotent stem cells (PSCs) were injected into Pdx1^{-/-} mouse blastocysts, (pancreatogenesis-disabled) and found that they developmentally compensated vacancy of the pancreatic “developmental niche,” generating almost entirely PSC-derived pancreas. The organ generation system described may be useful to treat organ failure in humans if pigs or other large animals are used.

However, several concerns have to be addressed first to bring this idea into practice. For example, if interspecific chimeras between mouse and rat were to be generated, their “embryonic lethality is high and maturation into adulthood is uncommon (Kobayashi et al., 2010).”

In another study the immune-deficient athymic nude mouse was used as a recipient of human tissue grafts, to generate human animal chimeras. Because this mouse lacks T cells, it allows for many types of xenograft tissues to survive and grow (Behringer, 2007). Four to seven grafting experiments using human fetal material in the nude mouse have been successful. In 1969 the first successful transplantation of human malignant tumor tissue in the nude mouse was reported. Since then, the number of tumors transplanted has increased steadily. The nude mouse with transplanted malignant tumors is a new alternative model in oncologic research. It can be applicable to: “basic studies of the genesis of cancer tissue, immunology, and cell kinetic research (Spang-Thomsen and Visfeldt, 1976).” The nude mouse was used as a model for cancer, by grafting pieces of human tumors under the skin of the nude mice, in that way, providing a “bio incubator” for tumor growth. This bio incubator gives scientists a glimpse into how to treat diseases in humans. The introduction of the nude mouse significantly enabled these types of studies and this in vivo assay is one of the fundamental experimental models for cancer research (Behringer, 2007).

Other experiments include studying mice with human germ lines. By subcutaneously transplanting human pre-pubertal and adult testicular tissue fragments into immune deficient mice, it is seen that human spermatogonia can survive in mice. Hopefully with more research, it may be possible to come up with a strategy to preserve the germline of young boys undergoing chemotherapies and radiotherapies that cause sterilization (Behringer, 2007). This study utilized Nude mice and severe combined immunodeficiency- non-obese diabetic mice, (SCID-NOD), two immunodeficiency recipients to compare the grafting of pre-pubertal and adult murine and adult human testicular tissue. The survival of pre-pubertal and adult murine testicular tissues, and of adult human testicular tissue was evaluated after subcutaneous grafting to immune deficient mice. This xenograft model in an immune deficient mouse with pre-pubertal human testicular tissue is a theoretical strategy for restoring fertility in childhood cancer patients, while circumventing the risk of malignant recurrence (Geens et al., 2006).

Ethical issues

These different types of human animal chimeras have provided important insights into fundamental biological mechanisms and the development of therapeutic protocols for human disease. Chimeras have compensated as human animal models for

the study of human processes in vivo. However there has been a lot of ethical debate concerning this research. These experiments reviewed have been approved, because the carefully transplanted human cells aren't large enough to make the animal human. They only humanize certain parts, and the resulting chimeras are euthanized after a number of days. Many boundaries had to be set with this type of research. In these studies, the human donor contribution is very low compared to the animal host. Although cells express an evidently higher degree of activity than genes, they also are intricately dependent on their surroundings. When hSCs are transplanted into animals, although they are free to proliferate and differentiate they are still in an animal body, and are reliant on their environments. They help to humanize an immune system if the animal is lacking one, however the host still remains an animal, with a distinct cell line of human cells, resulting in a chimera. Only in a receptive host, local niches regulate key developmental changes for several adult stem cell types, termed as an "inseparable relationship", besides for bone marrow transplantation, in which the hematopoietic stem cell is an "agent" in blood reconstitution (Hyun, et al 2007).

Research with human animal chimeras become a problem when cognitive capacities of the animal is changed, and when the germ line is affected. Therefore, boundaries have been put into place. Precautions in this field of research include, using progenitors rather than PSCs to avoid germ line contribution, which pose the risk of producing human embryos in animals. And treating the humanized mice, as a modified crop, by keeping it isolated to prevent it from mating with other mice, as well as euthanizing it immediately when research is concluded (Hermerén, 2015).

Conclusion

The above studies reviewed involved reconstituting animal models by transplanting human stem cells, HIV, cancer, and growing human organs in mice. The experiments resulted in a human animal chimera, an animal with two cell lines. Human animal chimeras, animals carrying human tissues were used as an alternative model to investigate human specific biological processes without experimentation on human individuals. Although human animal chimeras provide numerous possibilities, there is still a lot of controversy and risk associated with it. Since research is developing rapidly, and people's values and perception of risk and benefit change, not all issues can be settled. This approach to studying human pathways in animals concerns many people, however with the proper care and thought-out methods, research should be able to proceed without extreme regulation, and more experiments using human animal chimeras for therapeutic protocols should be approved.

Abbreviations

HSCs- Human Stem Cells
HESCs- Human Embryonic Stem Cells
hMNC's- Human Low-density Mononuclear cells
hUCB- Human Umbilical Cord Blood
PCR- Polymerase Chain Reaction
IHC-Immunohistochemistry
PB- Peripheral Blood
HIV-1-human Immunodeficiency Virus type 1 infection
BM- Bone Marrow
Alb-uPA- Albumin-Urokinase Plasminogen Activator
PSCs- Pluripotent Stem Cells
SCID-NOD- Severe Combined Immunodeficiency- Non-Obese Diabetic

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