BIOMEDICAL APPLICATIONS OF GENETICALLY ENGINEERED AND CLONED ANIMALS

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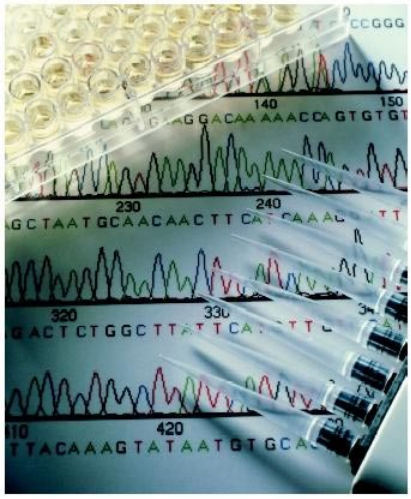
Biomedicine is the application of the principles of the natural sciences, especially biochemistry, molecular biology, and microbiology, to human and veterinary clinical medicine. The field of biomedicine offers tremendous potential to treat pain and suffering in humans and animals worldwide. Biomedical research using genetically engineered and cloned animals is being conducted to produce therapeutic drugs for the treatment of human diseases, for the production of organs for human transplantation, and to study the effects that individual genes on body function.

Scientists have used the techniques of cloning and genetic engineering as tools to advance this field of study. Often these terms are lumped together as being the same, but they are actually quite different. Cloning is the process of replicating an exact genetic copy of a plant or animal. On the other hand, genetic engineering is the introduction of DNA sequences into the genome of a living organism using naturally-occurring enzymes to "cut" a fragment of DNA from one organism and "paste" it into the genome of another. These terms are often affiliated with each other because cloning can be used as a vehicle to increase the efficiency of genetic engineering. Cloning allows for the production of animals from cells which have undergone precise, characterized modifications to their genome, or genetic background.



How can gene knockout be used to understand the function of genes?

To understand the function of individual genes, scientists use a process known as gene knockout, to disrupt the function of a gene of interest. In the laboratory, scientists can develop a non-functional copy of a gene of interest. When this non-functional copy is introduced to cells in culture, it can recombine and replace the functional copy, in a process called homologous recombination. Cells which contain the non-functional version of the gene of interest can then be used to produce a cloned animal which does not contain the functional version of the gene. Often these gene knockout studies are done in mice and other rodents to develop animal models for human disease. For example, to better understand how humans which suffer from cystic fibrosis are affected by the disease, mice have been genetically engineered so that the normal version of a gene, which does not cause cystic fibrosis, has been replaced with a mutated version.[1] The resulting 'knockout' mice display symptoms similar to humans with cystic fibrosis. Scientist can study how these mice are affected by the mutated version of the gene and use them to better understand how humans who suffer from cystic fibrosis might be better treated.

In addition to the use of gene knockout as disease models in rodents, it has also been used in livestock species. The prion protein, which is responsible for bovine spongiform encephalopathy (BSE; i.e. Mad Cow Disease) in cattle, has been knocked out[2,3]. BSE is a neurodegenerative disease which is thought to be correlated to a human disease called variant Creutzfeldt-Jakob disease[4,5]. Cattle in which the gene responsible for BSE has been removed could be used as a source of BSE-free tissues for use in human medicine.

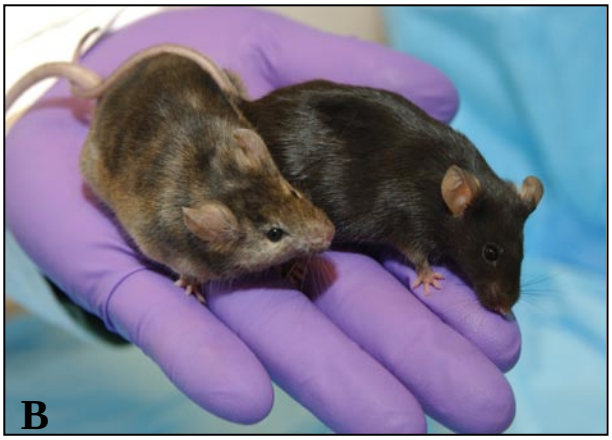


Figure 1. Genetically engineered animals which have had genes knocked out. A) Calves lacking the prion protein responsible for mad cow disease, B) the mouse on the left has had a gene which affects hair growth knocked out. Photos from Reicht et al (2006) and National Human Genome Research Institute, respectively.

Gene knockout has proved to be a very powerful technology. The impact that gene knockout has had on the understanding of single gene function is so significant that the 2007 Nobel Prize in Physiology or Medicine was awarded to the three individuals responsible for the discovery and development of gene knockout procedures (www.nobelprize.org).

How can genetic engineering and cloning be used to produce human therapeutic proteins?

Genetic engineering can be used to create animals which produce proteins that they would not normally be able to produce. Often these proteins are therapeutic proteins, which can then be used to treat disease in humans or animals. Human therapeutic proteins can also be produced in mammalian cell-culture based manufacturing facilities, but these facilities are expensive to construct, operate and maintain. The manufacturing capacity for therapeutic proteins cannot keep pace with the rapid progress in drug discovery and development, and this has resulted in unmet needs and dramatically rising costs. Genetically engineered mammals may provide an important source of these protein drugs in the future because the production of recombinant proteins in the milk and blood of transgenic animals presents a less-expensive approach to producing therapeutic proteins in cell culture. Mammals are of particular value in the production of human therapeutics proteins because they are able to make mammalian-specific protein modifications, such as adding specific sugar molecules to the transgenic protein, which can improve its stability and therapeutic efficacy.

Through genetic engineering, scientists can add a gene to a single cell, and then produce a cloned animal from that genetically engineered cell by placing its nucleus into an unfertilized egg which has had its own nucleus removed. The egg can then be activated and transferred into a surrogate female who will go through pregnancy and give birth to the cloned, genetically engineered animal. Human therapeutic proteins have been produced in rabbits[6], sheep[7,8], goats[9-12], pigs[13,14], and cattle[15-17]. In 2006, the first human therapeutic protein, Antithrombin III (ATryn®, GTC Biotherapeutics, Framingham, MA), derived from the milk of genetically engineered goats was approved by the European Commission for the treatment of patients with hereditary antithrombin deficiency[18,19].

Figure 2. Genetically engineered lysozyme goat. Photo by Alison Van Eenennaam, UC Davis.

Figure 3. Genetically engineered and cloned cattle at Hematech Inc. (Sioux Falls, SD). These cattle can be used for the collection of human polyclonal antibodies, which could then be used to fight disease in humans. Photo taken by Alison Van Eenennaam, UC Davis.

Further, Hematech Inc.(www.hematech.com), a biotechnology company in Sioux Falls, SD., has used both genetic engineering and cloning to create cattle which produce human polyclonal antibodies[20]. Hematech first used a 'gene knockout' technique to remove the genes responsible for the production of bovine antibodies from bovine cells in culture. These cells were then used to produce a cloned cow which could not manufacture bovine antibodies. Hematech then took cells from the cloned animal and inserted the human genes responsible for antibody production. These genetically engineered cattle cells carrying the human antibody genes, were then used to generate human polyclonal antibody-producing cattle. Upon immunization with a disease agent, these cattle are able to produce human antibodies that can be purified and used to treat that disease in human patients. Currently, the source of human polyclonal antibodies is from human volunteers who donate plasma, but the current supply cannot keep up with the demand. Production of human polyclonal antibodies in genetically engineered cattle would allow for the large scale production of antibodies. Antibodies are collected from the blood of transgenic cows through plasmapheresis, in much the same way as they are currently collected from human donors. Following purification, antibodies have the potential to be used to fight infections, assist humans with compromised immune systems, or to treat autoimmune diseases such as rheumatoid arthritis.

Human therapeutic proteins are also being produced in poultry. The production of therapeutic proteins in chickens offers a number of advantages. Firstly, their generation interval is short, which means that there is less of a time lag between the development of lines of genetically engineered poultry and the production of therapeutic proteins. Secondly, chickens produce a lot of protein in the eggs they lay, and those proteins can be purified from eggs using well-established protocols.

Origen Therapeutics, a company that specializes in the production of human therapeutics in chickens, recently developed chickens which produce human antibodies in the whites of the eggs they lay[21]. Similar to the antibodies produced in transgenic cattle, monoclonal antibodies isolated from genetically engineered chickens can be used to treat human disease.

Figure 4. Genetically engineered chicken. Photo from the Roslin Institute (UK).

AviGenics, another bio-pharmaceutical company, has developed a line of genetically engineered chickens which produce human interferon α-2b[22], which could be used to treat hepatitis C infection in humans. In fact, after recently receiving approval to test their product on humans, AviGenics has recently started human trials to determine how well their product works in human subjects[23].

What applications does genetic engineering and cloning have for xenotransplantion?

As of July 2008, nearly 100,000 Americans were on the organ transplant waiting list, with approximately 90% of candidates waiting for either a kidney or liver transplant (www.optn.org). Compounding this problem is the fact that allotransplantation, or the transplant of human organs into human recipients, cannot keep up with this growing list of recipients. Animal biotechnology offers a solution to this problem through xenotransplantation.

Xenotransplantation, which is the surgical transplantation of organs or tissues from one species to another, could be used to alleviate the demand for human organs for transplantation. For example, a liver from a pig could be transplanted into a human recipient. Pigs are considered a good species for xenotransplantation because their internal organs are approximately the same size as human organs. In addition, pig organs are physiologically similar to human organs.

However, one of the problems associated with using pig organs for xenotransplantation is that the immune system of the human recipient attacks the transplanted organ, causing transplant rejection. Pigs naturally produce a sugar, called α1,3-galactosyltransferase (αGalT), on the surface of their cells, which the human immune system recognizes as foreign. The human immune system then forms antibodies to attack the cells which produce that sugar, resulting in tissue rejection.

Through the use of genetic engineering and cloning, scientists have created pigs which are deficient for αGalT [24-26], and do not produce it on the surface of their cells. Transfer of these genetically engineered tissues and organs into baboon recipients has increased the length of time before the organs are rejected by the recipient's immune system[27-29]. Although further work needs to be done to enable the rejection-free transplant of organs from genetically engineered pigs to humans, it is envisioned that when organs from these genetically engineered pigs are transferred into human recipients, the human immune system will not recognize them as foreign, and so the organ rejection response will not be initiated.

Figure 5. Genetically engineered pig, suitable for xenotransplantation. Photo from World Health Organization.

Type of Transplantation	Transfer	Example	Pros	Cons
Allotransplantation	Within Species	Human – Human	Organ rejection less likely	Limited supply of organs
Xenotransplantation	Across Species	Pig - Human	Larger potential supply of organs	Higher rate of organ rejection

Table 1. Differences between allotransplantation and xenotransplantation.

Summary

Genetically engineered and cloned of animals have a variety of biomedical applications. These technologies may be used to develop animals which produce proteins and antibodies which are effective for the treatment of a variety of human diseases. In addition, they may also provide a safe and continuous supply of organs for xenotransplantation. Finally, it may be possible to produce livestock that are immune to certain diseases, or which are unable to produce proteins that are known to be deleterious to human health.

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