Genetic Modification of Swine for Agriculture and Medicine

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Introduction

The pig is an important component of the world's food supply. As of December 1, 2009 the hog inventory in the United States of America was 65.8 million head. The United States is the world's third largest producer and second largest consumer, exporter, and importer of pork and pork products. Not only are pigs important to agriculture, swine have become important in biomedical research as they are excellent models for cardiovascular disease (TurkLaughlin 2004), atherosclerosis (Ishii et al. 2006), cutaneous pharmacology (Herkenne et al. 2006), wound repair (Graham et al. 2000), cancer (Du et al. 2007), diabetes (Dyson et al. 2006), ophthalmology (Shatos et al. 2004), toxicology research, lipoprotein metabolism, pathobiology of intestinal transport, injury and repair, as well as being considered potential sources of organs for xenotransplantation (Lai et al. 2002). Furthermore, the swine genome is also quite similar to the human, as a phylogenetic approach using swine genome sequence data shows that the swine genome is 3x closer to the human than is the mouse (Wernersson et al. 2005). Reviewers at the National institutes of Health (NIH) consider swine to be a very important model for human health and disease conditions as evidenced by the fact that for the past 6 years extramural support of research on swine has averaged over $115 million per year (NIH Office of the Director). The NIH considers the swine to be so important that it has helped establish the National Swine Resource and Research Center at the University of Missouri (http://nsrrc.missouri.edu/) to serve as a genetic resource for the biomedical community.

Because the pig has such an important role in providing protein to the human diet, and because it serves as such a good biomedical model for humans, both anatomically and physiologically, there has been considerable effort to genetically modify pigs. While many genetic modifications have been made with little swine genomic sequence information, now that a first draft of the swine genome is publically available and additional sequence information is added on a daily basis the specificity of making both transgene additions and knock-outs/-ins is increased.

These genetic modifications have been made by pronuclear injection (Hammer et al. 1985), oocyte transduction (Cabot et al. 2001), somatic cell nuclear transfer (SCNT) (Park et al. 2001) or sperm mediated gene transfer (Lavitrano 1997). While the addition of a transgene is a very powerful tool, the ability to knock-out a gene opens up the possibility of even greater

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impact on understanding the genome of the pig. The rest of the text below will list most of the genetic modifications that have been made in pigs. The list will begin with some basic "tool" pigs and then move to medical applications, where the vast majority of modifications have been made, and finish up with agricultural applications and predictions of where the field is headed.

Types of genetic modifications

The types of genetic modifications that have been made to pigs are diverse and varied. They include both medical and agricultural applications. As a basic tool to create a marker for tracking cells, the enhanced green fluorescent protein has been added to pigs and then these fluorescent cells have been used to track the cell fate (Klassen et al. 2008; Price et al. 2006). Transgenes encoding other fluorophores (Matsunari et al. 2008; Webster et al. 2005) as well as LacZ (Jackson et al. 2009) have also been added and will likely have wide utility in the future. Control over the expression of transgenes in pigs has been demonstrated both with external control, as with the case of tetracycline (Kues et al. 2006) and with a molecular control as in the case of Cre expression (Li et al. 2009).

Xenotransplantation. Swine organs are very similar to humans in both size and function, and so probably the greatest efforts have been put forth to attempt to make pig organs transplantable to humans. The initial barrier is that of hyperacute rejection (HAR) caused by preexisting antibodies in the human that recognize a galactose α1,3 galactose residue on the surface of pig organs. Overcoming HAR was initially attempted by adding transgenes whose products would inhibit the compliment system, e.g. CD46, CD55, & CD59(Diamond et al. 1996, 2001; Langford et al. 1994). A second approach was to reduce the expression of swine galactose α1,3 galactose by overexpressing a transgene encoding α1,2-fucosyltransferase which utilizes the same substrate that produces galactose α1,3 galactose (Costa et al. 1999; Miyagawa et al. 2001). The next strategy involved disrupting or knocking out the gene (GGTA1) for the enzyme responsible for placing the offending galactose residues on the cell surface (Lai et al. 2002). This last approach was highly effective and broke down the wall of HAR (Kuwaki et al. 2005; Tseng et al. 2005; Yamada et al. 2005), only to reveal only more clearly the secondary response, acute vascular rejection (AVR).

As with HAR a number of approaches have been undertaken to address AVR. These include addition of transgenes for the human leukocyte antigen (Huang et al. 2006; Tu et al. 1999), HLA-E/h β2-microglobulin (Weiss et al. 2009), tumor necrosis factor-α-related apoptosis-inducing ligand (Klose et al. 2005), A20 zinc-finger (Oropeza et al. 2008), and CD39 (Ayares 2009). Non-vascular rejection is also a concern and the transgene for CTLA-4Ig, a human T-cell inhibitor molecule has been added to address this issue (Martin et al. 2005).

Overcoming organ rejection is not the only hurdle for successful xenotransplantation from swine to human. Another concern with xenotransplantation is the possibility of porcine endogenous retorviruses (PERVS) recombining in a human cell and creating a highly virulent strain of virus. To reduce that possibility inbred strains of pigs have been generated whose PERVS do not recombine with primate cells in vitro (Scobie et al. 2004; Simon et al. 2003), and a transgene has been introduced to knockdown PERVS (Dieckhoff et al. 2008).

While not considered xenotransplantation, transgenic pigs have been produced in which human liver cells can be developed for possible transfer to humans needing a new liver (Beschorner et al. 2003a,b, 2007), and pigs that make human albumin may provide a bridge, or artificial liver support, until a transplant becomes available (Naruse et al. 2005).

Models of Human Diseases. Since the pig's body size and physiology is similar to the human mimicking disease states in the pig permits invasive monitoring of the initiation and progression of disease. Thus creating a model for what occurs in humans provides physicians with something to both experiment upon and develop therapies. A number of such models have been created and include pigs that develop retinitis pigmentosa (Banin et al. 1999), diabetes (Renner et al. 2008; Umeyama et al. 2009), psoriasis (McCalla-Martin et al. 2009) and mammary tumors (Yamakawa et al. 1999). Attempts have also been made to create a model of Huntington's Disease (Uchida et al. 2001) and Alzheimer's Disease (Kragh et al. 2008), and to better study cardiovascular disease by modifying the composition of polyunsaturated fatty acids (Lai et al. 2006), or by reducing nitric oxide (Whyte et al. 2009). Probably the most significant modifications to create a disease model has been to disrupt the cystic fibrosis transmembrane conductance regulator (CFTR) gene to create a model of cystic fibrosis (Rogers et al. 2008). The cystic fibrosis model is especially important as the CFTR mutations introduced into mice have not resulted in a phenotype that mimics what occurs in humans. Remarkably, knocking-out the gene in swine has resulted in a recapitulation of almost all the symptoms that occur in humans with cystic fibrosis (Rogers et al. 2008).

Pharmaceuticals. A number of transgenes that encode proteins that could be used as pharmaceuticals have been produced in pigs. These have included human hemoglobin (Sharma et al. 1994) that might be used to treat trauma patients, Protein C as a coagulant (Van Cott et al. 1997), and both human coagulation factors VIII and IX (Lindsay et al. 2004; Paleyanda et al. 1997). While one may not immediately think of the pig as an animal that can produce milk from which these factors can be isolated, a small herd of pigs can easily provide the world's needs of pharmaceuticals such as coagulation factors VIII and IX (Van Cott et al. 2004). Another potential pharmaceutical that is being produced in pigs is human granulocyte-macrophage colony stimulating factor (Park et al. 2008).

Production Agriculture. Many transgenes that may affect the efficiency of producing meat or may alter the carcass composition of the pig have been introduced. These include human growth hormone (Hammer et al. 1985), insulin like growth factor 1 (Pursel et al. 1999), and Δ-12 fatty acid desaturase (Saeki et al. 2004). To make the pigs more resistant to heat stress heat shock protein 70.2 has been introduced (Chen et al. 2005). In an attempt to improve the prolificacy of sows Bcl-2 was introduced (Guthrie et al. 2005). Another interesting modification that may have a significant impact on production agriculture is the addition of a transgene for bovine α-lactalbumin (Bleck 1998). Transgenic sows produce more milk and thus wean heavier litters. Briefly mentioned above was a modification to study cardiovascular disease, i.e. the addition of the *hFat-1* gene (Lai et al. 2006). This gene converts Ω-6 fatty acids to Ω-3 fatty acids. While this modification would be useful to study cardiovascular disease, since Ω-3 fatty acids are limiting in the western diet, it might also become an important component of the human diet. Finally the gene for phytase was

introduced into pigs so that phytase produced by the salivary glands of the pig can breakdown inorganic phosphorus so that the pig can absorb it, rather than excreting it into the manure and contribute to pollution problems (Golovan 2001).

Conclusions and direction

Numerous genetic modifications have been made to pigs for biomedical purposes and undoubtedly will have an important impact on human health and medicine. In contrast to biomedicine, agricultural application of genetic modified animals are lagging behind and no transgenic animals are currently permitted to enter the food supply; even so, there are numerous potential applications of transgenic technology to generate new or altered strains of pigs. Particularly the interesting aspects of agricultural transgenics are the potential to increase disease resistance, improve carcass composition, improve feed usage, and enhance reproductive efficiency, etc. by introducing specific genetic modifications into pigs.

Considering that the efficiency of making transgenic animals remains relatively low, improvement of strategies for efficiently making transgenic animals includes transgene delivery, subtle genetic modification and novel methods of facilitating nuclear remodeling and reprogramming of somatic cells will be required. Major hurdles to overcome include the low rate of homologous recombination as a delivery method for transgenes or creation of knock-outs. This is particularly important in swine, as the donor fibroblasts for SCNT have a limited number of population doublings before senescence (Zhu et al. 2004). The utilization of emerging strategies such as zinc finger nucleases to introduce DNA double strand breaks, can dramatically increase the rate of recombination-mediated gene targeting by up to 1,000-fold (Moehle et al. 2007). Combining other technologies such as lentiviral vectors and RNAi for knocking-in and knocking-out gene function with somatic cell nuclear transfer have also shown promise in producing transgenic cells. The potential of pig induced pluripotent cells applied to transgenic technology in pig production and biomedicine are tremendous. The utility of this technology is limited only by our ability of identifying appropriate genes and gene functions to manipulate in the animal genomics.

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