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Genetic modifications of pigs for medicine and agriculture

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SUMMARY

Genetically modified swine hold great promise in the fields of agriculture and medicine. Currently, these swine are being used to optimize production of quality meat, to improve our understanding of the biology of disease resistance, and to reduced waste. In the field of biomedicine, swine are anatomically and physiologically analogous to humans. Alterations of key swine genes in disease pathways provide model animals to improve our understanding of the causes and potential treatments of many human genetic disorders. The completed sequencing of the swine genome will significantly enhance the specificity of genetic modifications, and allow for more accurate representations of human disease based on syntenic genes between the two species. Improvements in both methods of gene alteration and efficiency of model animal production are key to enabling routine use of these swine models in medicine and agriculture.

Keywords

transgenic; cloning; disease; model; swine; human

INTRODUCTION

The domestic swine genome has been altered through selective breeding to produce pigs with desired characteristics in agriculture for many centuries. The first “nontraditional” genetic modification in pigs was accomplished through microinjection of hundreds of copies of a foreign DNA fragment into the pronuclei of pig zygotes. The stable integration of a non-native gene into the swine genome resulted in the generation of the first transgenic pigs (Hammer et al., 1985). This ability to insert new genetic material and/or subsequently delete or replace genes opened new possibilities for the use of pigs as a research animal. The medical community had already accepted pigs as an excellent model for surgical testing based on their respective organs’ similarity to human heart, coronary vasculature, liver, kidney, lungs, and uterine histology (Swindle, 2007). The prospect of obtaining genetically modified pigs further extended their biomedical potential, especially to mimic inherited human diseases and to establish experimental DNA-altering treatments (i.e. gene therapy). Pigs are ideal for this type of genetic research as the size and composition of the porcine genome is similar to that of humans (Bendixen et al., 2010). The completed draft of the pig genome will be based on the most recent assembly (*Sus scrofa* genome build 10 (Sscrofa10); currently being annotated), which represents about 98% of the porcine genome (Groenen et al., 2011). The use of organs from transgenic pigs for xenotransplantation into humans (Lai et al., 2002a) and the production of pharmaceuticals (Park et al., 2006) are also being

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explored. From an agricultural standpoint, the worldwide demand for pork continues to rise as the populations and incomes of developing countries increase (United Nations FAO, 2009). Genetically altered swine have the potential to meet this demand by enabling development of strains with improved production traits, more efficient feed usage, increased reproduction, and greater protection from acquiring and transmitting disease.

This review covers, to the best of our knowledge, the various genetically modified pig models that have been produced to date. Improvements in methods to generate such swine has led to an expansive growth in the number of pig models specifically developed to enhance human health and nutrition. In addition to the genetically modified pigs described in the text of this review, several swine models are also listed in Table 1.

PRODUCTION OF TRANSGENIC PIGS

The first method to produce transgenic pigs, *pronuclear microinjection*, was originally established in mice (Brinster et al., 1981). DNA is microinjected into the pronuclei of zygotes collected from a superovulated female, and then transferred to a recipient pig by embryo transfer (Hammer et al., 1985). The technique is reliable and widely used, but only about 1% of injected eggs produce transgenic pigs (Niemann, 2004; Prather et al., 2008). An alternative method, *oocyte transduction*, uses a replication deficient virus to deliver the transgene to the porcine oocytes and can increase the percentage of transgenic offspring (Cabot et al., 2001; Hofmann et al., 2003). *Sperm-mediated DNA transfer* has also been used to produce genetically modified pigs with high efficiency based on the ability of sperm cells to internalize and integrate exogenous DNA during fertilization (reviewed in Lavitrano et al., 2005). Each of these methods can successfully make transgenic swine, but limitations include the inability to prescreen embryos for transgene integration prior to embryo transfer, the lack of expression specificity arising from random integration of foreign DNA, and the fact that only transgene addition is permitted, not deletion (i.e. gene knockout).

Targeted introduction of transgenes and loss-of-function mutations via homologous recombination in embryonic stem (ES) cells has been used for genetic manipulation of mice for decades (Capecchi, 2000). The lack of a stable source of true pig ES cells, however, has impeded the use of this method for creating chimeric, transgenic pigs with germline cells that participate in gamete formation (germilne transmission). One novel alternative approach is to graft transgenic male pig germ cells into immunodeficient mice (ectopic xenograft), resulting in the production of fully functional xenogeneic sperm for fertilization after intracytoplasmic sperm injection (ICSI; Honaramooz et al., 2008). This method is still in development, but viable non-transgenic piglets produced using sperm from ectopic testicular xenografts have been reported (Nakai et al., 2010). It remains to be seen how successful transgenic, ectopic xenografts will be in the production of genetically modified pigs.

The most popular method of producing genetically modified pigs to date is through genomic modification of somatic cells followed by nuclear transfer (NT), first reported by Park et al. (2001). In this process, the nuclei of somatic cells are transferred into enucleated metaphase II oocytes, and then this complex is activated by electrofusion. Reconstructed embryos are then cultured and transferred to synchronized recipients for gestation. The advantages of NT for gene transfer in pigs was described by Robl and First (1985), and nuclei from porcine blastomeres were used to produce the first cloned pigs from embryonic donor cells (Prather et al., 1989). Soon after the demonstration that the nuclei of adult somatic cells could undergo proper reprogramming to produce viable mammalian offspring (Wilmut et al., 1997), the first cloned pig derived from differentiated cells (porcine fetal fibroblasts) was reported (Onishi et al., 2000) and was soon followed by the cloning of pigs from cultured adult granulosa cells (Polejaeva et al., 2000). In the same year, Betthauser et al., (2000)

published significant procedural improvements to NT, including extension of the in vitro culture of fetal cells preceding nuclear transfer, in vitro maturation and activation of oocytes, and in vitro embryo culture.

As described in this review, the combination of genetically modified somatic pig cells and NT technology has produced many valuable biomedical and agricultural swine models. Despite these achievements, NT remains an inefficient process in pigs, as it does in all mammals without stably cultured ES cells. Many reconstructed pig embryos fail early in pregnancy, requiring large numbers of embryo transfers per recipient. The prevailing view is that incomplete epigenetic reprogramming of donor cell nuclei results in aberrant gene expression during development (reviewed in Zhao et al., 2010). Embryo culture conditions that alter epigenetic modifications of DNA, including histone deacetylase inhibitors (e.g. Trichostatin A, Scriptaid), can have a beneficial effect on the development of cloned pig embryos (Zhao et al., 2009; Martinez-Diaz et al., 2010). Pregnancy rates in recipients may also be improved by treating reconstructed embryos with the proteasome inhibitor MG132 after electrical fusion/activation (Whitworth et al., 2009). Transient treatment with MG132 likely prevents premature oocyte activation and lowers the rate of embryo fragmentation after NT (Sutovsky and Prather, 2004). Petersen et al. (2008b) found that by transferring cloned embryos immediately after activation to the oviducts of recipients that were 24 h asynchronous to the embryos increased pregnancy rate (9% with synchronous recipients versus 53% with asynchronous recipients) and survival of porcine embryos (1.8% vs. 12.5%). The authors hypothesize that this increased cloning success is related to the freshly reconstructed embryos having more time for proper genetic reprogramming. Additional optimized methods of swine genetic modification (Whyte et al., 2011a) and transfection (Ross et al., 2010) have reduced donor cell culture times in an effort to improve NT offspring viability. After transfer to recipients, inadequate placental development is frequently observed in NT-derived pigs (Niemann et al., 2008). Patterns of gene and protein expression in the extraembryonic membranes of NT pigs indicate improper development at the fetal-maternal interface (Whitworth et al., 2010), observations that imply a need for pharmacological interventions to improve development of the extraembryonic tissue. These advances have improved the ability to genetically modify porcine donor fibroblasts to produce cloned transgenic pigs to a state that a number of useful NT disease models exist, and are poised to have significant impacts in biomedicine and agriculture.

BIOMEDICAL APPLICATIONS

Cell Tracking

Some of the first “proof-of-principle” models of transgenic pigs included NT clones that expressed enhanced green fluorescent protein (eGFP) (Park et al., 2001; Lai et al., 2002b). Since that time, pigs that express red fluorescent protein (Matsunari et al., 2008) and multi-gene fluorescent proteins (blue, green and red) have been produced (Webster et al., 2005). Tissues from these transgenic swine are useful in biomedical research requiring genetically and phenotypically marked cells or organs. In one such study, cells isolated from fetal eGFP-transgenic pigs were used to evaluate the survival of porcine retinal progenitor cells as allografts in wild-type recipient pigs with damaged retinas (Klassen et al., 2008). Following transplantation, eGFP expression allowed histological visualization of cell integration in the recipient pigs, demonstrating the utility of these transgenic pigs as a large animal model.

Pig-to-Human Xenotransplantation

The first landmark swine model derived specifically for human health was in the field of xenotransplantation. The urgent need for replacement organs is unmet by human organ donations, with over 110,460 recipient candidates awaiting organ transplants in the United

States (United Network for Organ Sharing, 2011). This has spurred a large scientific effort to make pig organs transplantable to humans. Rejection of pig organs by human recipients is caused by humoral and cell-mediated responses (reviewed in Klymiuk et al., 2010). Hyperacute rejection (HAR) occurs within 24 hours of transplantation and results from antibody recognition of galactose α1,3-galactose (α-Gal; epitopes synthesized by the enzyme α-1,3-galactosyltransferase (GGTA1). Acute humoral xenograft rejection (AHXR), usually begins after the first week of transplantation, and is caused by mismatched human leukocyte antigen (HLA) complex antigens that are present on all cells. To circumvent HAR, swine fibroblasts were produced with a knockout of the gene GGTA1. Production of cloned swine from these fibroblasts was a major victory in the search for human organ replacements (Lai et al., 2002a). To augment protection against HAR, cloned GGTA1 knockout swine were produced with a transgene overexpressing α1,2-fucosyltransferase, which uses the same substrate that produces α-Gal, but instead produces a universally tolerated human antigen (Ramsoondar et al., 2003). Antibody-initiated complement activation is one dominant mechanism through which AHRX occurs. Transgenic cloned swine that overexpress the complement regulatory protein human-decay accelerating factor (hDAF) and an enzyme that modifies α-Gal, N-acetylglucosaminyltransferase III (GnT-III) have been developed to protect against AHXR and HAR, respectively (Fujimura et al., 2004). Swine with multiple genetic modifications (e.g. GGTA1 knockout combined with hDAF overexpression) appear to be the most promising route to widespread availability of organs for human transplant.

All pig breeds carry porcine endogenous retroviruses (PERVs) due to their integration in the swine genome (Patience et al., 2001). Although no known swine disease has been definitively attributed to PERVs, elevated viral expression in melanomas and pulmonary metastasis-derived cell cultures from selectively bred Munich miniature swine has been observed (Dieckhoff et al., 2007). The function of the virus in tumour development is still unclear. The greater concern for PERVs is the risk of infection of human cells during xenotransplantation of pig organs to humans, presenting a major hurdle to the use of pig organs in human recipients. To inhibit infection of human cells with PERVs, Dieckhoff et al. (2008) transfected porcine fibroblasts with a lentiviral vector expressing a short hairpin RNA (shRNA). The expression of a specific shRNA initiates the RNA interference (RNAi) cascade (Whitehead et al., 2009), prompting a significant inhibition of PERV expression in cloned piglets. If this level of PERV inhibition is sufficient to prevent human infection, these pigs may be combined with other genetically modifications described previously to advance successful organ xenotransplantation.

Cardiovascular Disease

Swine are historically considered an excellent model for the human cardiovascular system (Turk and Laughlin, 2004). As a potential dietary source of essential and beneficial fatty acids, genetically modified pigs have been developed to express Δ12 fatty acid desaturase (FAD2) from spinach (*Spinacia oleracea*; Saeki et al., 2004) to increase linoleic acid, as well as pigs expressing a humanized *Caenorhabditis elegans* gene, fat-1, encoding an *n*-3 fatty acid desaturase to increase the *n*-3:*n*-6 fatty acid ratio in meat (Lai et al., 2006). These pig models can also be used to examine the cardiovascular effects of an altered *n*-3:*n*-6 fatty acid ratio in the swine themselves. Another important regulator of vascular health is the signaling molecule, nitric oxide (NO). Transgenic swine that overexpress endothelial nitric oxide synthase (eNOS; Hao et al., 2006; Whyte and Laughlin, 2010), responsible for producing NO in the inner lining of blood vessels, will increase our understanding of the complex regulation of vasodilation and potential therapies for diseases related to endothelial dysfunction. Vascular signaling by NO is closely associated with endothelial hydrogen peroxide (H_2O_2), a molecule that plays key roles in cardiovascular regulation (Drouin and

Thorin, 2009) and age-accelerated vascular disorders (Collins et al., 2009). To further explore the vascular role of H_2O_2, transgenic Yucatan minipigs that overexpress human catalase in the endothelium were developed (Whyte et al., 2011b). These large-animal cardiovascular models will enable real-time measurement of functional parameters including blood flow, temperature, tissue oxygenation, perfusion and diffusion that are difficult or impossible to monitor in similar genetically modified rodent models.

Cystic Fibrosis

Cystic fibrosis (CF) is an autosomal recessive disease affecting about 30,000 individuals in the United States (reviewed in Messick, 2010). CF is caused by mutations in the gene that encodes for the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel. Symptoms of CF arise from faulty water and ion transport across epithelial tissues, leading to inadequate hydration of mucous secretions. These thick secretions in organs such as the lungs initiate progressive cycles of infection and inflammation, the main cause of morbidity and mortality. Over 1600 mutations related to CF have been described, and although several CFTR knockout mouse models have been developed, none of them develop the obstructive lung disease symptoms seen in humans (Bragonzi, 2010). In an effort to produce an accurate CF model, pigs were cloned from fetal fibroblasts in which the CFTR gene was mutated to produce null and ΔF508 (the most common genetic mutation in CF) alleles (Rogers et al., 2008; Welsh et al., 2009). These newborn CF pigs display defective chloride transport, meconium ileus, pancreatic destruction, and early focal biliary cirrhosis that occur in newborn humans with CF (Meyerholz et al., 2010). In contrast to CF mouse models, these CF pigs develop the principal characteristics of human CF lung disease (Stoltz et al., 2010; Ostedgaard et al., 2011). Recently, the phenotype of CF pigs resolved a long-misunderstood question about the order of events in the inflammation/infection pathogenic cycle. The initial event displayed in CF pigs is impaired bacterial elimination from the lungs, followed by a cascade of inflammation and pathology (Stoltz et al., 2010). This key finding about CF pathogenesis provides new options for CF therapy and prevention.

Alzheimer's disease

Alzheimer's disease (AD) is a progressive manifestation of dementia that typically begins with subtle and poorly recognized failure of memory, gradually becoming more severe, and eventually, incapacitating the patient (reviewed in Avramopoulos, 2009). The genetic basis for familial, autosomal dominant AD (FAD) lies in mutations to the genes PSEN1, PSEN2, and the amyloid precursor protein gene (APP). These mutations are associated with increased production of proteolytic fragment Aβ which aggregates into fibrils and toxic oligomeric forms, initiating synaptic damage and neurodegeneration (Walsh et al., 2005). A genetically modified pig model for FAD was developed based on similarities in size, physical features, and rate of growth between the porcine and human brain (Kragh et al., 2009). Porcine fibroblasts hemizygous (carrying a single copy) for a neuron-specific splice variant of human APP were generated via transfection with a cDNA construct, followed by NT cloning. This splice-variant carries an AD-causing dominant mutation known as the Swedish mutation (Kragh et al., 2009). The transgene was expressed in the brain, but the authors speculate that it may take until the age of 1–2 years before Aβ accumulates to symptomatic levels in the porcine brain (Götz and Götz, 2009). Most (95%) AD cases of later onset, however, do not follow Mendelian inheritance, despite showing significant heritability (Avramopoulos, 2009). Pig models that address the genetic foundations of other forms of AD could provide translational data for early detection and new treatment in patients.

Spinal muscular atrophy

Spinal muscular atrophy (SMA) is an autosomal-recessive neurodegenerative disease, and the most common genetic cause of infant mortality (reviewed in Prior, 2010). Clinical manifestations of SMA include motor neuron loss and skeletal muscle wasting. Spinal muscular atrophy (SMA) is caused by a deletion or mutation of the survival motor neuron (SMN1) gene. A highly conserved gene (SMN2) is also present exclusively in humans. Mutations in SMN2 have no clinical consequence if SMN1 is retained. In the case where SMN1 is mutated, the disease severity correlates inversely with SMN2 gene copy number, although SMN2 alone cannot prevent the disease.

A recent porcine animal model for SMA was developed specifically to evaluate the efficacy, pharmacology, and toxicology of SMA therapeutics (Lorson et al., 2011). The authors highlighted the importance of demonstrating the conservation of alternative splicing events of both human SMN1 and SMN2 in the pig in order to produce a successful transgenic model of SMA. The porcine model of human SMA is being produced in three stages: First, a knockout of the SMN allele produced $SMN^{+/-}$ pigs. Next, a human SMN2 transgene is being added to the pig genome (currently in progress at the time of this review). Finally, $SMN^{-/-}$; SMN2 pigs will be generated through breeding and a second round of nuclear transfer (Lorson et al., 2011). Nuclear transfer of SMN targeted fetal fibroblasts produced healthy $SMN^{+/-}$ piglets that, like their human $SMN^{+/-}$ counterparts, are phenotypically normal. Efforts are underway to introduce the human SMN2 transgene into swine fetal fibroblasts, leading to completion of the pig model of SMA. Effective SMA therapeutics do not currently exist, highlighting the value of a genetically modified pig model for this disease.

Diabetes

Another transgenic swine model for human disease is for the phenotype of diabetes mellitus (Umeyama et al., 2009). Diabetes mellitus (DM) is defined by the American Diabetes Association as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (reviewed in Raslova, 2010). Type 1 DM results from autoimmune destruction of β-cells in the pancreas, while Type 2 DM is a generalized metabolic disorder resulting from reduced tissue sensitivity to insulin. Chronic hyperglycemia caused by absent or ineffective insulin is associated with long-term damage to organs, especially the eyes, kidney, nerves, heart and blood vessels. Type 2 DM represents more than 80% of the DM cases, and approximately 57 million Americans are classified as “prediabetic” (Pour and Dagogo-Jack, 2010). Type 3 DM is a maturity-onset diabetes of the young (MODY3), which is an early-onset non-insulin-dependent type of diabetes characterized by autosomal dominant inheritance.

A transgenic pig model for Type 3 DM was produced based on a mutation in the human hepatocyte nuclear factor 1α (HNF-1α) gene (Umeyama et al., 2009). The mutated HNF-1α construct was delivered by ICSI-mediated gene transfer followed by NT. Although 22 live transgenic piglets were farrowed, close to 80% of these clones died within 10 days of birth. The role of transgenesis and the cloning procedure in this mortality could not be determined. The surviving piglets, however, exhibited a diabetic phenotype, including high blood glucose and poor insulin secretion from the Islets of Langerhans. Although pig models of diabetes have previously been generated by chemical destruction of the β-cells, these cloned pigs form the first transgenic model where pigs exhibit the pathophysiological characteristics of diabetes. More recently, transgenic pigs expressing an impaired glucose-dependent insulinotropic polypeptide (GIP) receptor were generated via lentiviral transgenesis (Renner et al. 2010). Normally, insulin secretion is induced by GIP resulting from hyperosmolarity of glucose in the duodenum. This pig model exhibited significantly

reduced oral glucose tolerance, demonstrating an essential role of GIP for insulin secretion. Additionally, proliferation of β-cells and physiological expansion of β-cell mass were significantly reduced in these transgenic pigs, closely resembling the characteristic features of human type 2 diabetes.

Pharmaceutical Production

The high cost of purified proteins required to treat diseases like hemophilia limits treatment in most developed countries, and is prohibitive in developing nations (Fischer et al., 2002). The predominant methods of isolation of proteins from human plasma donations or from transgenic proteins produced in mammalian cell culture are costly and inefficient. For instance, only 10% of factor VIII present in one liter of donated plasma can be isolated (Mannucci, 2004). As an alternative, pig mammary bioreactors would be an excellent system for large-scale production of therapeutic proteins due to the high volume of milk that can be harvested per day (2–3 liters) and the quality of post-translational processing of complex proteins (Van Cott et al., 2004). It is estimated that the at the amount of transgenic protein isolated per milliliter of porcine milk, only 60 transgenic pigs would be required to supply the *entire* amount of factor IX needed in the United States (Van Cott et al., 2004). To put this in perspective, to isolate the same quantity of protein, 1,200,000 liters of plasma would be required, the equivalent of approximately 1,600,000 plasma donations.

There are several reports of using pigs to produce human recombinant proteins, by either pronuclear injection or NT, such as human factor VIII (Paleyanda et al., 1997) and IX (Van Cott et al., 1999), hemoglobin (Swanson et al., 1992), human protein C (Van Cott et al., 2001), human erythropoietin (Park et al., 2006), human granulocyte-macrophage colony stimulating factor (Park et al., 2008), and von Willebrand factor (Lee et al., 2009). For such proteins to be used as replacements in human patients, herds of transgenic swine must be isolated in pathogen-free facilities, and there must be routine testing of isolated protein lots for quality and purity (Van Cott et al., 2004).

AGRICULTURAL APPLICATIONS

Meat production, disease resistance, and efficient use of feed are the areas of agriculture that stand to benefit most from genetically modified swine production. The use of genetically modified pigs as a source of higher quality, more efficiently produced food with reduced environmental impact tends to fall under greater governmental and public scrutiny than the biomedical models previously discussed. This is likely due to the fact that biomedical pig models are restricted to the laboratory, whereas transgenic pigs in agriculture would present direct entry of transgenic material into the human food chain. It is prudent to assess the safety of any product entering the marketplace for human consumption, but genetically modified crops and animals are held to a higher approval standard (for recommendations, see FDA-CVM, 2009). It is agreed that the risks must be addressed, but the assessment should constitute a *true* risk-benefit analysis (as outlined by Murray and Maga, 2010) that considers the risk, if any, against the potential benefits and the cost to the consumer, the producer, the environment and to the animals themselves if the technology is *not* used.

Meat Production

Early work by Pursel et al. (1990) showed that genes for growth-related hormones (e.g. growth hormone, insulin-like growth factor) could be expressed in pigs produced by DNA microinjection. These transgenic pigs had markedly reduced subcutaneous fat and variable growth enhancement relative to control pigs, but also exhibited problems such as fatigue, gastric ulcers, and low libido (although fertile). These effects were suggested to be due to

the growth hormone produced, and suggestions were made to improve the health of the pigs generated.

Myostatin (MSTN) is the only secreted protein demonstrated to negatively influence muscle mass in vivo (Long et al., 2009). Targeting this gene could result in enhanced muscle growth in pigs, yielding more lean meat per animal. To date, no myostatin knockout pigs have been generated, but porcine fetal fibroblasts transfected with a MSTN targeting vector have been reported to have significantly lower expression of MSTN mRNA compared to control fibroblasts (Li et al., 2009). This suggests the potential for cloned MSTN-knockout pigs derived from these fibroblasts to have more muscle growth than wild-type pigs.

Enhanced production can also be achieved by improving the milk quality and/or amount available to newborn pigs. Wheeler and colleagues (2001) produced transgenic sows that express bovine α-lactalbumin in the mammary gland, which leads to an increase in milk production. Weight gain was higher in piglets suckling from alpha-lactalbumin gilts as compared to piglets suckling from controls.

Disease and Stress Resistance

Model pigs with enhanced defenses against pathogenic or environmental stresses are few, at present, but genetic modification could improve survivability and ultimately meat quality. Under high heat conditions, finishing pigs have reduced growth performance and poorer carcass characteristics due to their limited thermoregulation capacity (Spencer et al., 2005). To improve pig thermotolerance, transgenic pigs overexpressing porcine heat shock protein HSP70.2 were produced by pronuclear microinjection (Chen et al., 2005). The survival rate of primary fibroblast cells under 45°C culture conditions was significantly higher from the transgenic pig compared to the non-transgenic pig. The authors speculate that transgenic pigs overexpressing HSP70 might be more resistant to summer temperatures, thereby reducing mortality and economic loss in animal production.

Feed Efficiency

Increasing the absorption of nutrients from pig diets has the dual benefit of efficient food usage while reducing manure-based environmental pollution. The majority of phosphorus in livestock grain feed is in the form of natural phytate, a more stable and complex phosphorus compound that pigs are not able to digest completely, leading to large quantities of phosphorus in the pig manure (Emiola et al., 2009). To address these concerns, Golovan et al. (2001) developed transgenic pigs by pronuclear microinjection that express a protease-resistant *Escherichia coli* phytase transgene in the pig salivary gland. The transgenic phytase functions under the low-pH conditions of the pig digestive tract. Variability in phytase activity among transgenic pig lines was attributed to the positional effect of the transgene insertion. This genetic modification resulted in essentially complete digestion of dietary phytate and reduced fecal phosphorus excretion by up to 75%. Transgenic model pigs such as this present a new biological approach for reducing waste/pollution in swine farming and decrease the need for supplemental nutrients to be added to feeds.

CONCLUSION

The number of genetically modified pigs produced in the last 15 years has grown exponentially as techniques have been standardized and become globally accessible to more researchers. The combination of emerging technologies for gene modification with the completion of the swine genome will bring to reality model pigs that can provide a safe, reliable source of urgently needed organs and therapeutic proteins for humans, and provide realistic representations of human disease. Production pigs that can adapt to a variety of

environmental conditions while being less susceptible to acquiring and/or transmitting disease will help to meet the nutritional demands of the ever-expanding world population. Surely, the greatest advances from genetically modified pigs will arise from ideas and concepts not yet envisioned.

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Abbreviations

ES	embryonic stem
ICSI	intracytoplasmic sperm injection
NT	nuclear transfer

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Table 1

Genetically modified pigs for use in biomedicine and agriculture.

Use / Modification	Issue Addressed / Transgene Activity	Reference
Xenotransplantation		
membrane cofactor protein, MCP (CD46) $^{+}$	Hyperacute rejection: Complement Modifier	(Diamond et al., 2001)
human-decay accelerating factor, hDAF (CD55) $^{+}$	Hyperacute rejection: Complement Modifier	(Langford et al., 1994)
CD59^{+} (MIRL)	Hyperacute rejection: Complement Modifier	(Fodor et al., 1994)
α-1,2-fucosyltransferase (FUT2) $^{+}$	Hyperacute rejection: Carbohydrate remodeling	(Koike et al., 1996)
β-1,4- acetylglucosaminyltransferase (MGAT2) $^{+}$ *	Hyperacute rejection: Carbohydrate remodeling	(Miyagawa et al., 2001)
α-1,3-galactosyltransferase (GGTA1) $^{-/-}$	Hyperacute rejection	(Lai et al., 2002a)
heme oxygenase I (HMOX1) $^{+}$	Post-Hyperacute rejection	(Petersen et al., 2008a)
ecto-ATPase (CD39) $^{+}$	Post-Hyperacute rejection	(Dwyer et al., 2007)
thrombomodulin (THBD) $^{+}$	Post-Hyperacute rejection	(Petersen et al., 2007)
human leukocyte antigen, DP (HLA-DP) $^{+}$	Cell Mediated rejection	(Tu et al., 1999)
human leukocyte antigen, DR (HLA-DR) $^{+}$	Cell Mediated rejection	(Huang et al., 2006)
human leukocyte antigen, E (HLA-E) $^{+}$ / B2M $^{+}$	Cell Mediated rejection	(Weiss et al., 2009)
tumor necrosis factor ligand superfamily, member 10 (TNFSF10) $^{+}$	Cell Mediated rejection	(Klose et al., 2005)
tumor necrosis factor-alpha-induced protein 3 (TNFAIP3) $^{+}$	Cell Mediated rejection	(Oropeza et al., 2008)
Fas ligand (FASLG) $^{+}$	Cell Mediated rejection	(Choi et al., 2010)
cytotoxic T lymphocyte-associated 4 (CTLA4) $^{+}$	Non-Vascular rejection	(Martin et al., 2005)
Belatacept (LEA29Y)	Non-Vascular rejection	(Klymiuk et al., 2009)
porcine endogenous retroviruses (PERV) knock-down shRNA	Porcine Endogenous Retrovirus	(Dieckhoff et al., 2008)
Liver Transplantation		
alpha-ferroprotein cytosine deaminase (AFP-CDA) $^{+}$	Liver specific expression of cytosine deaminase	(Beschorner et al., 2003a)
albumin thymidine kinase 1 (ALB-TK1) $^{+}$	Liver specific expression of thymidine kinase	(Beschorner et al., 2003b)
Liver Support		
albumin (ALB) $^{+}$	Artificial liver	(Naruse et al., 2005)
Immune Function		
immunoglobulin kappa, constant region (IGKC) $^{-/-}$	Delete light chain expression	(Ramsoondar et al., 2010)
immunoglobulin heavy chain, joining gene cluster (IGHJ) $^{-/-}$	Delete antibodies and B cells	(Mendicino et al., 2010)
Disease Model		
rhodopsin, mutant P347L (RHO P347L) $^{+}$	Retinitis Pigmentosa	(Petters et al., 1997)
rhodopsin, mutant P23H (RHO P23H) $^{+}$	Retinitis Pigmentosa	Ross et al. unpublished

Use / Modification	Issue Addressed / Transgene Activity	Reference
cystic fibrosis transmembrane conductance regulator (CFTR) $^{+/-}$ and CFTR$^{\Delta F508/+}$	Cystic Fibrosis	(Rogers et al., 2008)
Huntington (HTT) $^{+}$	Huntington's Disease	(Uchida et al., 2001)
omega 3 fatty acid desaturase $^{+}$	Cardiovascular Disease	(Lai et al., 2006)
catalase (CAT)$^{+}$	Cardiovascular Disease	(Whyte et al., 2011b)
endothelial nitric oxide synthase 3 (NOS3) $^{+}$	Cardiovascular Disease	(Whyte et al., 2010)
proprotein convertase, subtilisin/kexin-type, 9 (PCSK9) $^{+}$	Familial Hypercholesterolemia	(Bolund et al., 2010)
apolipoprotein E (APOE) knock-down shRNA^{+}	Familial Hyperlipidemia	(El-Beirouthi et al., 2009)
gastric inhibitory polypeptide receptor, dominant negative (GIPR(dn)) $^{+}$	Diabetes	(Renner et al., 2010)
hepatocyte nuclear factor-1 homeobox A, dominant negative (HNF1A(dn)) $^{+}$	Diabetes	(Umeyama et al., 2009)
insulin 2, mutant C93S (INS2 C93S) $^{+}$	Diabetes	(Renner et al., 2010)
amyloid precursor protein (APP) K670N^{+}/M671L^{+}	Alzheimer's Disease	(Kragh et al., 2008)
mouse mammary tumor virus (MMTV)/v-Ha-ras $^{+}$	Mammary Tumors	(Yamakawa et al., 1999)
survival motor neuron (SMN) $^{+/-}$	Spinal Muscle Atrophy	(Lorson et al., 2011)
Pharmaceuticals		
protein C (PROC) $^{+}$	Protein C and anticoagulation	(Van Cott et al., 1997)
Factor VIII (FVIII) $^{+}$	Human coagulation Factor VIII	(Paleyanda et al., 1997)
Factor IX (FIX) $^{+}$	Human coagulation Factor IX	(Lindsay et al., 2004)
hemoglobin B, beta (HBB) $^{+}$	Human hemoglobin beta	(Sharma et al., 1994)
colony-stimulating factor 2 (CSF2) $^{+}$	Human granulocyte-macrophage colony-stimulating factor	(Park et al., 2008)
Production Agriculture		
growth hormone (GH) $^{+}$	Growth hormone	(Hammer et al., 1985)
lactalbumin (bLALBA) $^{+}$	Alpha-lactalbumin	(Bleck et al., 1998)
insulin-like growth factor 1 (IGF1) $^{+}$	Increase muscle mass	(Pursel et al., 1999)
Sloan-Kettering viruses (c-Ski) $^{+}$	Increase muscle mass	(Pursel et al., 1992)
phytase^{+}	Metabolize inorganic phosphorus	(Golovan, 2001)
delta 12 fatty acid desaturase^{+}	Spinach gene to change body composition	(Saeki et al., 2004)
heat-shock protein 70-KD protein 2 (HSPA2) $^{+}$	Create animals resistant to heat stress	(Chen et al., 2005)
B-cell leukemia 2 (BCL2) $^{+}$	Increase ovulation rate	(Guthrie et al., 2005)
myostatin pro-domain^{+}	Increase muscle mass	(Mitchell and Wall, 2008)
Tool Pig		
enhanced green fluorescent protein (eGFP) $^{+}$	Cell Tracking	(Cabot et al., 2001; Park et al., 2001)
enhanced blue fluorescent protein (eBFP) $^{+}$, DsRed2 $^{+}$	Cell Tracking	(Webster et al., 2005)
Kusabira-orange^{+}	Cell Tracking	(Matsunari et al., 2008)
tetracycline-inducible human-decay accelerating factor (TET-hDAF)	Tetracycline dependent expression	(Kues et al., 2006)

Use / Modification	Issue Addressed / Transgene Activity	Reference
[+], tetracycline-inducible CD95 (TET-CD59) [+]		
octamer-binding transcription factor 4 (Oct4)-eGFP [+]	Germline-specific eGFP expression	(Nowak-Imialek et al., 2009)
β-galactosidase (LacZ) [+]	Cell Tracking	(Jackson et al., 2010)
eGFP- proteasome subunit, alpha-type, 1 (eGFP-PSMA1) [+]	Green labeling of proteasomes	(O'Gorman et al., 2010)
Technique Demonstration		
knock out eGFP	KO of eGFP by zinc-finger nucleases	(Whyte et al., 2011a)
α-1,3-galactosyltransferase (GGTA1) [−/−]	KO of GGTA1 by using zinc-finger nucleases	(Hauschild et al., 2010)
phosphoglycerate kinase-eGFP (PGK-eGFP) [+]	Lental virus vectors	(Hofmann et al., 2003)
human-decay accelerating factor (hDAF) [+]	Sperm mediated gene transfer	(Lavitrano, 1997)
enhanced green fluorescent protein (eGFP)[+]	Oocyte transduction	(Cabot et al., 2001)
cytomegalovirus enhancer/chicken beta-actin promoter::Venus (CAGGS-Venus)[+]	Sleeping beauty transposon-mediated transfection	(Garrels et al., 2010)
PiggyBac-mediated transposition	Tetracycline-dependent expression of RFP	(Kim et al., 2010)
retinoic acid 8-stimulated mitochondrial-localized enhanced yellow fluorescent protein (STRA8-mEYFP)	Retinoic acid inducible expression	(Sommer et al., 2010)

[*] UDP-N-acetylglucosamine:alpha-6-D-mannoside-beta-1,2-N-acetylglucosaminyltransferase II