17 Pigs as a Model for Biomedical Sciences

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Introduction: Creating the Building Blocks – Genomics, Transgenesis and Cloning

Obtaining a complete draft of the pig genome sequence has been central to the development and broad acceptance of the pig as a biomedical model (Schook *et al*., 2005a,b). The pig genome sequence has recently been completed (http://www.ensembl.org/Sus_scrofa/Info/Index), and the key building blocks for full utilization of the pig as a biomedical model are now in place: completed genome sequence, ability to produce transgenic animals and the ability to replicate the model through somatic cell cloning (Schook *et al*., 2005b). The emergence of genetic information and the development of the necessary tools to target manipulations, in combination with the ability to clone pigs, provide a new and highly relevant animal model. These building blocks have stimulated the development of 'genomic postulates' (Table 17.1) for evaluating animal models and, relevant to this chapter, the significance of the pig. This chapter was developed to provide background on the need for relevant animal models and to address each of the aspects of the genomic postulates. Owing to the overwhelming physiological (Tumbleson and Schook, 1996) and genomic similarities between pigs and humans (Humphray *et al*., 2007), the pig provides a uniquely relevant animal model for human disease. In addition, a recent CRISP (Computer Retrieval of Information on Scientific Projects) search (1999–2003) indicated that the US National Institutes of Health (NIH, which has over 20 institutes and centres) sponsored research that supported 2400 separate grants that utilized the pig. Thus, a broad foundation supporting the pig as a model in biomedical research already exists from which to build future programmes. There is also growing

Table 17.1. Genomic postulates, adapted from Koch's postulates.

1. Isolate and propagate causal gene from animal
2. Characterize (manipulate) gene *in vitro*
3. Reintroduce putative gene (create transgenic animal) to test causality
4. Demonstration of causal relationship through induced phenotype

interest within the biomedical community with respect to the utilization of pigs in bioengineering, imaging and behavioural studies.

The Animal Model Concept

The use of animals to study human physiology and anatomy can be traced back to the second century common era (CME) in which Galen, a Greek physician and philosopher, completed research studies on apes and pigs (Galen, 1586) (Fig. 17.1). Galen incorrectly assumed that all extracted information derived from his use of animals could be directly applied to humans. It was not, however, until the 16th century CME that his error was initially recognized (Nomura *et al*., 1987), when Bernard proposed the use of chemical and physical induction of disease in animals, thus becoming the first advocate for creating 'induced animal models' for biomedical research. At the turn of the 20th century came the development of infectious disease animal models and their use for evaluating antibacterial drugs, and the introduction of the 'germ theory of disease' (Koch, 1884; Fanning, 1908). The end of the 20th century and the beginning of the 21st century realized the ability to utilize naturally occurring models resulting from spontaneous mutations – severe combined immunodeficiency (SCID) or nude mice – and from genetically modified animal genomes through transgenesis or site-directed homologous recombination. Linkage with the ability to clone animals, either through the utilization of embryonic stem cells or somatic cell nuclear transfer, provided even further ability to use animals which have phenotypic characteristics close to humans as relevant animal models for dissecting human disease. Finally, the emergence of the whole genome sequencing of animals with many physiological similarities to the human, such as the pig, supports the ability to actually create a large animal model that is

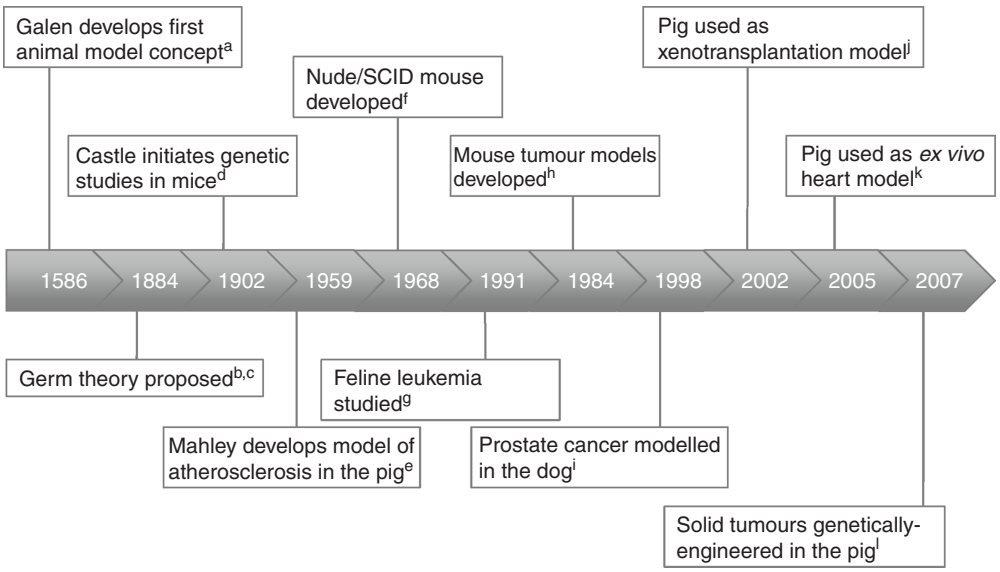

Fig. 17.1. Timeline of animal models. SCID, severe combined immunodeficiency. Sources: [a]Galen, 1586; [b]Fanning, 1908; [c]Koch, 1884; [d]Dunn, 1965; [e]Mahley *et al*., 1975; [f]Pantelouris, 1968; [g]Hardy *et al*., 1981; [h]Brinster *et al*., 1984; [i]Waters *et al*., 1998; [j]Cooper *et al*., 2002; [k]Laske *et al*., 2005; [l]Adam *et al*., 2007.

genetically and phenotypically similar to humans in terms of disease attributes.

Animal models represent important tools for investigating the pathogenesis of human disease and developing appropriate treatment strategies. The coupling of genomic information (genome sequence, gene expression profiling and proteomics) with enabling technologies (transgenesis and cloning) has revolutionized the development of human biomedical animal models. Traditionally, the mouse has been a powerful experimental system for understanding the complexity of cancer, diabetes and cardiovascular disease, among others. The dog is also considered a comparable model to human disease because of its similarities to human anatomy and physiology, particularly with respect to the cardiovascular, urogenital, nervous and musculoskeletal systems. As such, it has long been used as a model in drug discovery and development research. Human disease may best be recapitulated in a large mammal such as the pig. The pig is often the primary biomedical model for a number of diseases, for surgical research and for organ transplantation owing to the similarity in size, anatomy and physiology between pigs and humans (Swanson *et al*., 2004). Animal models, regardless of species, can be grouped into one of the following five categories: (i) spontaneous models; (ii) genetically modified models; (iii) induced or experimental models; (iv) negative models; and (v) orphan models (Table 17.2).

One approach to studying human disease is to characterize a naturally occurring disease in an animal that corresponds to a human disease. The best-known spontaneous model is the athymic nude mouse, the use of which represented a turning point in the study of heterotransplanted tumours and enabled the first description of natural killer cells (Pantelouris, 1968). Genetically engineered models were created that harboured genetic changes commonly found in human disease. The first transgenic mouse tumour model was established by overexpression of viral and cellular oncogenes in specific tissues (Brinster *et al*., 1984; Stewart *et al*., 1984; Adams *et al*., 1985; Hanahan, 1989). Induced models involve healthy animals in which the condition to be studied is experimentally induced through surgical modifications, genetic modifications, or chemical application – demonstrated in 1918 when Yamagiwa and Ichikawa showed that coal tar experimentally applied to rabbit ears caused skin carcinomas (Yamagiwa and Ichikawa, 1918). More recently, considerable insight has been gained into the strengths and weaknesses of toxicity and

Table 17.2. ntages and disadvantages of animal model types.

Model type	Advantages	Disadvantages	Examples
Spontaneous	Similar disease phenotype to humans	Long latency	Nude/severe combined immunodeficiency (SCID) mice (Pantelouris, 1968)
		Not genetically defined	Canine haemophilia (Giles, 1982); canine prostate cancer (Waters *et al*., 1998)
Genetically modified	Defined genetic background	Phenotypic expression of genes can differ	Porcine tumour model (Adam, 2007)
		Transgenesis and homologous recombination	Mouse tumour model (Brinster *et al*., 1984)
Induced or experimental	Gene expression controlled through diet or inducers	Not predictive of therapeutic success	Atherosclerosis (Mahley *et al*, 1975; Bell and Gerrity, 1992; Dixon *et al*. 1999)
	Rapid disease onset		Obesity (Spurlock and Gabler 2008)
	Free choice of species		Diabetes (Mordes and Rossini, 1981; Larsen *et al*., 2002; Larsen and Rolin 2004)
Orphan	Useful for evaluation of chemical/radiological treatments	Do not faithfully mimic human disease	Feline leukaemia (Hardy *et al*., 1981); bovine leukosis (Gillet *et al*., 2007)

carcinogenicity studies in laboratory rats and mice. Infectious disease models are often restricted to a limited number of susceptible species, and the remaining unresponsive species are considered negative models because they do not develop the disease when exposed to a particular stimulus (Hau, 2008). The main application of negative models is to gain insight into the physiological basis of disease resistance. There are functional disorders present in non-human species that have not yet been described in humans. Often, a similar disease will be identified in a human that was previously described in animals. These animals represent 'orphan models' for that particular disease as no human equivalent has been identified. Feline leukaemia (FeLV) represents a naturally occurring disease in domestic cats that is not transmissible to humans; like lymphoma in humans, lymphoma induced by FeLV in cats is characterized by immunosuppression.

The incidence of chronic disease due to complex genetic and environmental interactions, however, has continued to increase during the past century. Understanding human disease is difficult owing to the complexity of genetics and lifestyle interactions, and the high cost associated with developing therapeutics. As such, appropriate biomedical models are essential because most medical knowledge, treatment regimes and medical device developments are based on robust animal models. As genomic and bioinformatic technologies continue to advance, our knowledge of animal models will increase, thereby refining our choice of models and enabling the development of more applicable models. Animal models are essential tools for studying gene–gene interactions and gene–environment effects, and for preclinical testing of therapeutic interventions. Given that mice, the most common animal model, frequently do not faithfully recapitulate human disease, pigs will continue to serve as important biomedical models.

Utilizing the Pig to Improve Human Health

During its multiple domestication events, the pig has undergone intense selection pressures for various phenotypes throughout the world (Chen *et al*., 2007). First domesticated in Asia from the wild boar, germplasm was quickly moved around the world by explorers and used for food and products. Intense selection and breeding has provided distinct phenotypes differing in metabolism, fecundity, disease resistance and meat products (Schook *et al*., 2005b; Schook 2007). Such selective pressures have resulted in differentiated subpopulations and phenotypes extremely relevant to current and future human health research. The selection of 'mini' and 'micro' pigs for size, independently by investigators throughout the world, attests to the global relevance of this experimental animal in biomedical research. The porcine model is also relevant to human health research priorities such as obesity, female health, cardiovascular disease, nutritional studies (as the pig is an omnivore), and communicable diseases (reviewed in Tumbleson and Schook, 1996). The pig provides a valuable biological model in these priority areas because of the vast amount of research that has been conducted on the genetic and environmental interactions associated with complex, polygenic physiological traits.

Informing Human Physiology: Similarities between Pig and Human Phenotypes

Animal physiology has significantly contributed to the basic understanding of human development and physiology related to disease (Table 17.3). For example, classical endocrinology studies in pigs has led to the current understanding of several reproductive and pituitary hormones, most notably the composition of insulin, which was first determined for porcine insulin and was used for several decades to treat human diabetes (Rohrer *et al*., 2003). The porcine biomedical model has provided a fundamental research platform for developing human reproductive techniques and for studying reproductive diseases. Ongoing research using the pig to study cancer and diabetes is contributing greatly to our understanding of these diseases and is further expanded upon in this chapter (Table 17.3). The pig has many similarities in structure and

Table 17.3 ated swine biomedical models.

Type of investigation	Model	Reference
Heart physiology	Stent design, tissue engineering of blood vessels	Bedoya *et al.*, 2006; Gyöngyösi *et al.*, 2006
	Atherosclerosis	Turk and Laughlin, 2004; Turk *et al.*, 2005
	Myocardial infarction	Ambrose, 2006; Boluyt *et al.*, 2007
	Ex vivo heart model	Laske *et al.*, 2005
	Emergency procedures	Casas *et al.*, 2005; Geddes *et al.*, 2006
Reproductive function	Maternal–fetal interactions	Green *et al.*, 2006
	Embryo development	Sun and Nagai, 2003; Rohrer *et al.*, 2006
	Sperm	Strzezek *et al.*, 2005; Lavitrano *et al.*, 2006
Transplantation	Cell and organ transplants	Larsen and Rolin, 2004; Street *et al.*, 2004
	Xenotransplantation	Cooper *et al.*, 2002; Ibrahim *et al.*, 2006
Skin physiology	Percutaneous permeation	Simon and Maibach, 2000; Dalton *et al.*, 2006
	Contact dermatitis	Stuetz *et al.*, 2006
	Skin culture model	Huang *et al.*, 2006
	Melanoma	Geffrotin *et al.*, 2004; Zhi-Qiang *et al.*, 2007
Brain	Stroke	Imai *et al.*, 2006
	AIDS, dementia	Tambuyzer and Nouwen, 2005
	Drug-binding sites and interactions	Minuzzi, *et al.*, 2005
Gut physiology and nutrition	Gut structure and intestinal metabolism	Eubanks *et al.*, 2006; Qiu *et al.*, 2006
	Obesity	Brambilla and Cantafora, 2004
	Probiotics and gut physiology	Reid *et al.*, 2003; Domeneghini *et al.*, 2006
	Food allergies	Bailey *et al.*, 2005; McClain and Bannon, 2006
Biochemical	Response to injury	Schmitt and Snedecor, 2006
	Imaging techniques	Ellner *et al.*, 2004; Goldberg *et al.*, 2004
	Osteoporosis, bone density analysis	Teo *et al.*, 2006
Tissue engineering	Cartilage repair	Chang *et al.*, 2006
	Spinal fusion	Drespe *et al.*, 2005
	Organ-specific gene delivery	Kawashita *et al.*, 2005
	Cataract repair	Lassota *et al.*, 2006; van Kooten *et al.*, 2006
	Polymer scaffolds	Brown *et al.*, 2006; Moroni *et al.*, 2006
	Tooth development	Hu *et al.*, 2005
Respiratory function	Neonatal respiratory distress	Miller *et al.*, 2006
	Asthma	Turner *et al.*, 2002; Watremez *et al.*, 2003
Infectious disease	Therapeutics (vaccines, biotherapeutics, drug therapies)	González *et al.*, 2004; Cheetham *et al.*, 2006
	Developmental interactions	Hasslung *et al.*, 2005; Butler *et al.*, 2006
	Mucosal tissue responses	Elahi *et al.*, 2005; Dawson *et al.*, 2005; Pomeranz *et al.*, 2005; Dvorak *et al.*, 2006
	Host response	Houdebine, 2005

function to humans, including size, feeding patterns, digestive physiology, dietary habits, kidney structure and function, pulmonary vascular bed structure, propensity to obesity, respiratory rates and social behaviours (Tumbleson and Schook, 1996). Because the pig is an omnivore, it provides an adaptable model to evaluate chronic and acute exposures to xenobiotics such as alcohol, tobacco, feed additives and environmental pollutants (Schook, 2007). Pigs have been used as models to evaluate alcoholism, total parenteral nutrition, organ

transplantation, atherosclerosis, exercise, hypertension, melanoma, nephropathy, dermal healing, shock and degenerative retinal diseases.

A severe shortage of organs and tissues for transplantation has also stimulated increased consideration of pigs as a potential solution, particularly with the recent ability to genetically modify pigs to overcome acute rejection (Lai *et al*., 2002). Targets for the genetic modification of pigs for xenotransplantation have thus far emphasized reducing the immunogenicity of porcine cells and tissues, and preventing rejection after transplantation of porcine tissue. Acute rejection is mediated through preformed antibodies against galactosyl-α-1,3-galactose epitopes expressed on the surface of pig cells. Transgenic pigs have been developed that express regulators of the complement cascade, including CD55, CD59 and CD46, which suppress the attack on donor tissues (Bucher, *et al*., 2005; Cox and Zhong, 2005; Houdebine, 2005). Another approach has focused on eliminating the galactosyl-α-1,3-galactose antigen from the surface of donor cells. Researchers have generated pigs without the gene encoding α-1,3-galactosyltransferase (Zhong, 2007). This was accomplished by the serial knockout of the gene in cultured pig fibroblasts, followed by somatic cell nuclear transfer to generate pigs. The convergence of transgenic and cloning techniques has enabled multilayered genetic modifications to be made in a single animal.

Breeding among multiple existing transgenic lines and introducing new genes by somatic cell nuclear transfer can be used in combination to overcome the various stages of xenograft rejection associated with xenotransplantation (Matsunari and Nagashima, 2009). The necessary genetic modifications are dependent on the specific transplant procedure. For example, the removal of the αGal epitope to prevent antibody reactivity and the insertion of complement regulators would increase the success of vascularized grafts, while pancreatic islet grafts would require the insertion of complement regulators, anticoagulants to prevent an inflammatory reaction and an anti-apoptotic gene to counteract ischaemia and reperfusion injuries (d'Apice and Cowan, 2009). Using these approaches, polytransgenic and α-1,3GalT-KO pigs have been produced, but further research is needed to created an efficient model (Rood *et al*., 2005; Tseng *et al*., 2005; Yamada *et al*., 2005; Cooper *et al*., 2007).

Phenotypic research utilizing unique pig breeds has identified genetically controlled differences in fat deposition (Rothschild and Ruvinsky, 1998; Malek *et al*., 2001a,b). Such information provides the basis for developing an experimental model for understanding obesity and for the development of nutritional interventions from prenatal nutrition to aged cohorts. Porcine resource populations have been selected for phenotypic variation in bone density (osteoporosis), sex-expressed nutritional and reproductive characteristics, and growth and development (embryonic, prenatal and postnatal). Using comparative genomics, new models have been identified to study how metabolism is linked to obesity-induced diabetes (Milan *et al*., 2000). The porcine model will also be invaluable to study host–pathogen interactions for food safety (i.e. *Salmonella*), potential biological warfare agents (African swine fever; foot-and-mouth disease) and agents that affect food security and human health (i.e. porcine endogenous retroviruses and other zoonotic diseases).

Linking Genotypes and Phenotypes Relevant to Human Health

The discovery that mammalian genomes probably contain only 20,000–30,000 genes suggests that alternative transcripts and post-translational modifications must play a greater role in phenotypic expression than previously appreciated. It is also expected that single gene products affect different traits or disease states depending on the temporal and spatial presence of gene products. As an omnivore, the pig is prone to many of the same dietary health problems as humans. Depending on diet and genetics, pigs can suffer from hypertension, hypercholesterolaemia, dyslipidaemia, insulin resistance and atherosclerosis. The pig has mutations in similar genes affecting these metabolic disorders (i.e. *ApoB* and *LDLR* for hypercholesterolemia) (Ajiello *et al*., 1994; Hasler-Rapacz *et al*., 1998). Piglets are the preferred model organism to develop human

infant formula as their nutritional needs are comparable to those of human infants. Because of their similar digestive tracts, pigs are also susceptible to comparable enteric food-borne pathogens (i.e. *Salmonella*, enterohaemorrhagic *Escherichia coli*) and pig intestinal linings are used for *in vitro* studies of interactions with the intestine and these pathogens. Pigs are also susceptible to gastric ulcers that apparently are induced by diet and stress (Engstrand *et al*., 1990). Additional anatomical similarities with humans include renal morphology, eye structure, skin and tooth development. The pig is also one of few animals that will voluntarily eat to obesity, as well as being susceptible to alcoholism.

There are two reasons for research to investigate obesity-related genes in the pig. First, as already mentioned, the pig is a more realistic model organism for human obesity owing to its physiological similarities to humans (Tumbleson and Schook, 1996). As the pig is a true omnivore, the molecular basis and digestive tract anatomy of the pig is much closer to that of humans than any laboratory animal species, as identified by significant DNA polymorphisms of obesity-related genes in the pig genome that might provide useful targets for the genetic study of human obesity. The second reason is that the genetic components of human obesity can play important roles in pig performance traits such as fatness, growth rate and feed intake.

Surrogate Systems for Human Experimentation

The domesticated pig has provided numerous surrogate experimental models for biomedical research. There has been a long tradition of using abattoir tissues for the purification of enzymes and the elucidation of metabolic pathways. These tissues have also served as initial biologicals, with bovine and porcine insulin providing pre-recombinant DNA therapeutics and purified enzymes used to determine crystalline structure. Porcine gamete biology has played a critical role in our understanding of stem cells and *in vitro* fertilization (Wu *et al*., 2001; Yin *et al*., 2002). Because of the wealth of biological information derived from the porcine system, it has increasingly become important for studying epigenetic effects, as well as unravelling genomic imprinting. The demonstration that pigs can be cloned using *in vitro* cloning systems provides an invaluable technology platform for developing relevant clones of genetic models for biomedical research (Betthauser *et al*., 2000; see Chapter 11). In addition, a major obstacle for producing cloned genetically modified pigs has been overcome (Lai *et al*., 2002). Investigators have created a nuclear transfer technology using clonal fetal fibroblasts as nuclear donors for the production of gene-specific knockouts. This technology platform has significant applications beyond xenotransplantation, and the availability of genomic sequences will facilitate the broader utility of the pig as a surrogate system for human experimentation.

The phenotypic diversity of hundreds of porcine breeds distributed throughout the world provides a tremendous resource for 'comparative phenomics', the application of comparative genomic principles to the discovery of new genes underlying diverse phenotypes. In only a few thousand years, selective breeding has produced pig breeds that thrive in diverse environments (e.g. high altitude versus tropical), convert energy to muscle mass efficiently and rapidly, and tolerate specific pathogens. There can be little doubt that the understanding of what makes porcine breeds different with respect to reproductive efficiency, bone structure, growth rates, fat deposition, altitude or heat tolerance and resistance to specific pathogens will be important to understanding basic biological processes important to human health (see Chapter 18).

Extrapolation from Animals to Humans

The selection of an animal model depends on a number of factors relating to the hypothesis to be tested. Often a number of different models may advantageously be used to study a biological phenomenon associated with a human disease. For diseases such as cancer, there are a wide range of well-described models available,

both induced and spontaneous, in a variety of species. The key factor in using animal models for studying disease is that the results can be extrapolated of the humans. Animal models of human disease are deemed relevant only if they are useful in recapitulating disease pathogenesis and assisting in the development of approaches to intervention or therapy (Hau, 2008). Thus, to ensure full utilization, a model needs to reliably mimic the normal anatomy and physiology of human organs and tissues of interest, as well as accurately reflect the morphological and biochemical aspects of disease pathogenesis.

The rationale behind extrapolating results from an animal model to humans is primarily based on the similarity between morphological structures and physiological processes. For example, an animal model of cancer should ideally undergo tumour development and progression in a similar fashion to humans. While many animals are more or less similar to humans in regard to biological characteristics, there are prominent differences in body size between species, which affects their appropriateness as a model for certain experiments. The validity of extrapolation may be further complicated by the prevalence of disease in humans, with certain sectors of the population having a higher incidence of a type of disease over another owing to genetic and environmental influences.

Traditionally, animal models were used to identify the genes responsible for a disease. Trends in the use of animal models are changing as new technologies are enabling researchers to use animal models to study the effects of changes in genetic pathways. Developments in the fields of genomics, proteomics, biotechnology and bioinformatics are changing the nature of biomedical research. The Human Genome Project is providing genetic information, not only from humans, but also from animals traditionally used as models. Increased insight into genetic pathways and gene–environment interactions that are involved in the aetiology of complex human genetic disease are providing the knowledge required to select better animal models. This knowledge can be applied to produce specific transgenic animals or knockouts, which better mimic the physiological complexity of human disease than existing models. New, more precise models for the development of therapeutics can be created. Animal models are essential tools for studying gene–gene interactions and gene–environment effects, and for preclinical testing of therapeutic interventions.

An important theme in toxicology research is the search for and the assessment of animal models that are predictive for adverse effects of pharmaceuticals in humans. This process is based on the assumption that the current choice of animal models is truly predictive of a human response to a treatment. To validate this assumption, a large multinational pharmaceutical company survey analysed data compiled from 150 compounds to determine the concordance of the toxicity of pharmaceuticals observed in humans with that observed in experimental animal models (Olson *et al.*, 2000). The concordance rate was found to be 71% for comparable target organs in rodent and non-rodent species, with non-rodents alone being predictive for 63% (primarily the dog) of human toxicity and rodents alone for 43% (primarily the rat). The highest incidence of overall concordance was seen in haematological, gastrointestinal and cardiovascular human toxicities, and the least was seen in cutaneous human toxicity. The results of this survey support the value of *in vivo* toxicology studies to predict for human toxicity associated with pharmaceuticals, and indicate that data collected from experiments in animals can be extrapolated to humans. It can also be concluded that the type of animal model chosen must be carefully evaluated. Traditionally, toxicology studies utilize rat and dog models, without considering whether there is an alternative species that might be more appropriate for testing a specific compound. While no animal model can completely recapitulate the effects of every drug administered to humans, previous research has shown that large animals are better preclinical models for drug toxicity than rodents (Olson *et al.*, 2000).

Modelling Human Disease in the Pig

The pig has been used as an important large animal model for human disease for decades. The animal has a long lifespan of 10–15 years (Hau and Van Hoosier, 2003), so disease

progression is more similar to that seen in humans. Furthermore, as already discussed, the pig shares anatomical and physiological characteristics with humans that make it a unique and viable model for biomedical research (Tumbleson and Schook, 1996). Because of the similarity in body mass of pigs to humans, the pig has become a model of choice for tissue engineering and imaging studies (Lunney, 2007). Their large size also makes them ideal models for study in such medical fields as surgery, imaging, chemotherapy and radiation, which cannot be accurately tested in small animal models.

Their cardiovascular anatomy and physiology, in combination with the pig's response to atherogenic diets, have made them a universally standard model for the study of atherosclerosis, myocardial infarction and general cardiovascular studies. Their gastrointestinal anatomy has some significant differences from that of humans; however, the physiology of their digestive processes has made them a valuable model for digestive diseases. The urinary system of swine is similar to humans in many ways, especially in the anatomy and function of the kidneys (Swindle and Smith, 2000). Swine are also a standard model for skin and reconstructive surgical procedures, and have been developed as models of transdermal toxicity. The anatomy and physiology of organs such as the liver, pancreas, kidney and heart have also made this species the primary species of interest as organ donors for xenograft procedures (Swindle and Smith, 2000).

In addition, the ability to use pigs from the same litter, and cloned or transgenic pigs, facilitates genetic mapping (Lunney, 2007) and minimizes immunological differences between animals in transplant studies. The availability of numerous well-defined cell lines from a broad range of tissues will assist in studies of gene expression and drug susceptibility testing. Sequencing of the swine genome (Schook *et al*., 2005a) has provided increasingly advanced genetic and proteomic tools for pigs. Many of these studies employ genomic approaches, as in heart, transplantation and melanoma models. The pig genome has a high sequence homology to humans, 60%, compared with a 40% sequence homology of rodents to humans (Thomas *et al*., 2003; Humphray *et al*., 2007), and the pig chromosomal structure has a higher similarity to humans than those of the mouse, rat, dog, cat or horse, or cattle (Meyers *et al*., 2005; Murphy *et al*., 2005). Each model will be affected by the availability of the functional genomic tools, and swine genome sequence and maps (Rothschild *et al*., 2007; Tuggle *et al*., 2007).

Creating a Porcine Cancer Model

The pig is an attractive model to study cancer biology and to help close the gap between basic science and patient benefit. Compared with rodents, the pig is metabolizes drugs and undergoes tumorigenesis in a manner analogous to humans. Like humans, the incidence of cancer in pigs is rare, with a prevalence of childhood cancer – Wilm's tumours in young pigs (Anderson and Jarrett, 1968), and a broader spectrum of cancers in adults (Brown and Johnson, 1970). Furthermore, the pig provides an ideal system for preclincial studies of imaging, as well as of hyperthermia, radiation or photodynamic therapy of tumours. It is almost impossible to do intensity-modulated radiation therapy on mice owing to the small tumour size and the energy of the clinical accelerator. High-resolution intensity treatment in other rodents is hindered by the same problems, and devices used for hyperthermia treatment of tumours cannot be scaled down to be useful for studies in rodents.

Parallels in cancer biology between pigs and humans extend to the molecular level, as demonstrated by the reduced number of genes required to convert human and pig cells to a tumorigenic state compared with mouse cells (Kendall *et al*., 2005). Additionally, telomerase is suppressed in a number of tissues and reactivated during cancer in both humans and pigs (Pathak *et al*., 2000; Stewart and Weinberg, 2000), indicating that there are also similarities in the process of tumorigenesis between the species. The genomic sequence homology between pigs and humans is also very high (Swanson *et al*., 2004), and the porcine pregnane X receptor protein that regulates p450 cytochrome CYP3A, which metabolizes almost half of prescription drugs in humans, is more similar to that of humans than, for example, mice (Xie and Evans, 2002; Pollock *et al*., 2007).

It has been demonstrated that the enforced expression of transgenes that mimic genetic changes occurring in many types of human cancers can drive normal primary porcine cells to a tumorigenic state. Specifically, co-expression of human *TERT* (*hTERT*), $p53^{DD}$ (a dominant-negative truncation mutant of p53), *cyclin D1*, $CDK4^{R24C}$ (an activated version of a cyclin-dependent kinase 4 mutant), $c\text{-}Myc^{T58A}$ (a stabilized version of the oncogene c-Myc) and $H\text{-}Ras^{G12V}$ (a constitutively active form of Ras GTPase) have the ability to drive porcine fibroblasts to form tumours when explanted into immunocompromised pigs at different anatomical sites (Adam *et al*., 2007). These same genetic changes drive human kidney cells, mammary epithelial cells and myoblasts to a tumorigenic state (Kendall *et al*., 2005) indicating that tumorigenesis in pigs is similar to the process in humans. Genetically engineered porcine tumour cells provided the first method of inducing tumours in a large animal, and hence it is possible to tailor-make tumours of a defined background using the pig. Although this model is limited because the animals need to be immunosuppressed for tumours to grow (akin to xenograft mouse models), pigs nevertheless have a number of clear advantages that make them ideal for preclinical studies of human cancers. The resultant tumours in the pigs could be grown to very large sizes, ideal for a number of preclinical applications. This model can be exploited in different cell types to generate many different types of tumours potentially anywhere in the body (Table 17.4).

Emerging Cancer Models Utilizing the Pig Phenotype

Basal cell carcinoma is the most prevalent human cancer, with over 750,000 cancers being diagnosed yearly in the USA alone, yet animal models remain limiting owing to molecular and skin type differences between humans and mice. While mouse skin and human skin share many similar features, there are also major differences, which may contribute to the differences in skin tumorigenesis with respect to tumour type and mechanism between the two species. In humans, the three main types of skin cancer are: basal cell carcinomas (BCC), squamous cell carcinomas (SCC) and cutaneous melanomas (CM), with BCC being the most common of the three, representing approximately 70% of all human skin cancers (de Gruijl *et al*., 2001). In contrast, mice do not develop BCC; the predominant malignant tumour type in mice is SCC (Peto *et al*., 1975; Bogovski, 1994). In addition, oncogenic *Ras* has an essential role in mouse skin tumorigenesis while it appears to have only a minor role in human skin cancer (Ananthaswamy and Pierceall, 1990; Pierceall *et al*., 1991a,b). Thus, mice are not always ideal *in vivo* models for the study of human skin cancer.

Among experimental animals, porcine skin is most similar to human skin and has been used extensively as a model of human wound healing (Lunney, 2007). More specifically, the porcine integument is morphologically (Montagna and Yun, 1964; Meyer *et al*., 1978; Monteiro-Riviere and Stromberg, 1985; Monteiro-Riviere, 1986), histochemically (Meyer *et al*., 1986; Rigal *et al*., 1991; Woolina *et al*., 1991), biochemically and biophysically similar to human skin. As such, the pig has been utilized as a model for drug toxicity and percutaneous absorption studies. Pig skin resembles human skin in having a sparse hair coat, a relatively thick epidermis, and similar epidermal turnover kinetics, lipid composition, carbohydrate biochemistry, lipid biophysical properties and arrangement of dermal collagen and elastic fibres (Weinstein, 1966; Forbes, 1967; Montagna, 1967; Meyer *et al*., 1981; Meyer *et al*., 1982). Reported differences in pigs include a unique interfollicular muscle that spans the triad of the hair follicle (Stromberg

Table 17.4. [illegible]ne cell transformation.

Embryonic layer	Cell type transformed	Experimental model	Tumour type induced
Endoderm	Keratinocytes	*In vitro* cell transformation	N/A
Ectoderm	Fibroblasts; mammary, kidney and testes cells	*In vitro* cell transformation	Squamous cell carcinoma
Mesoderm	T cells	Live virus injection	T cell lymphoma

et al., 1981), the presence of apocrine sweat glands only on the body surface (Montagna and Yun, 1964; Monteiro-Riviere and Stromberg, 1985), and a thicker stratum corneum (Meyer *et al*., 1978; Bronaugh *et al*., 1982). With regard to biochemical similarities between pigs and humans, for example, conservation of the matrix methalloprotease genes *MMP1* and *MMP9* is greater between humans and pigs (89% and 85%, respectively) than between humans and mice (80% and 78%, respectively, based on the HomoloGene NCBI (US National Center for Biotechnology Information) database).

As discussed previously, it has been demonstrated that porcine fibroblasts can be transformed *in vitro* and explanted into the pig to form tumours. Fibroblasts, however, are the primarily transformed cell type in less than 1% of human malignancies (Khavari, 2006). BCC, the most common cancer in the USA, and SCC, the second most common cancer in the USA, arise from keratinocytes (Khavari, 2006). Isolated porcine keratinocytes, the target cell population, can be transformed following the same procedure. Specifically, the co-expression of *hTERT*, $p53^{DD}$, *cyclin D1*, $CDK4^{R24C}$, $c\text{-}Myc^{T58A}$ and $H\text{-}Ras^{G12V}$ is sufficient to drive porcine keratinocytes to form tumours when injected subcutaneously into immunocompromised mice. Further research has demonstrated that expression of only *cyclin D1*, $CDK4^{R24C}$, $H\text{-}Ras^{G12V}$ and $c\text{-}Myc^{T58A}$ was sufficient to transform both porcine fibroblasts and keratinocytes to a tumorigenic state, indicating that fewer genes are required for successful porcine cell transformation and subsequent tumour formation (K.N. Kuzmuk, 2009, unpublished results).

The establishment of tumours using the pig as a model is possible, provided the animals remain on immunosuppressive therapy. When treatment with immunosuppressive drugs is halted, tumours, regardless of size, regress owing to an overwhelming host immune reaction to the tumour cells. Research using retroviruses as vectors is being conducted to determine whether this approach eliminates the need for immunosuppressed animals. It is theorized that the manipulation of cells in tissue culture during the transformation process makes the cells immunogenic. It has been demonstrated that the injection of a virus encoding mutated H-Ras directly into the mammary fat pads of wild-type rats is tumorigenic (McFarlin and Gould, 2003; McFarlin *et al*., 2003). For that reason, the direct *in vivo* injection of retroviruses containing the transgenes required for porcine cell transformation *in vitro* would be tumorigenic in immunocompetent pigs. To test this hypothesis, viruses expressing the transgenes used to transform both the porcine fibroblasts and keratinocytes (*cyclin D1*, $CDK4^{R24C}$, $H\text{-}Ras^{G12V}$ and $c\text{-}Myc^{T58A}$) were injected directly into the pig. Direct retroviral injection produced a low frequency of lymphoma of T cell origin (K.N. Kuzmuk, 2009, unpublished results).

Needs and Opportunities for Expanding the Use of Pig Biomedical Models

Novel approaches to harvesting genomic information to target genetic manipulations coupled with cloning have been identified as targets for further development (Schook *et al*., 2005b). Emerging technologies such as recombineering and gene trapping combined with relevant, standardized cell lines of targeted modifications could be used for cloning specific pigs for a given human disease. The National Swine Resource and Research Center (NSRRC) at the University of Missouri (http://www.nsrrc.missouri.edu) provides essential support for creating genetic pig models of human diseases. Specifically, NSRRC has established significant resources to assist researchers in creating transgenic pigs, as well as to support the distribution of created models to investigators, thus, providing a mechanism for generating and distributing the 'gold standard' model for specific diseases or phenotypes.

Finally, the pig will continue to grow as the biomedical model of choice in bioengineering and experimental surgery, and in zoonosis research related to the emergence of new diseases such as swine influenza. With respect to bioengineering and experimental surgery, the growing popularity of the pig versus the dog has continued to rise, and the pig is now the most common large laboratory animal species. The number of pigs used in 2002 in registered research facilities as reported to

the US Department of Agriculture (USDA) was over 68,400, whereas the number of dogs declined from 201,000 in 1984 to 68,200 in 2002 (http://www.aphis.usda.gov/publications). Completion of the pig genome sequencing will only accelerate the popularity and value of swine in biomedical research. The pig is currently being developed as a model to understand the pathogenesis of and immunity to human viral pathogens such as rotavirus, calicivirus and coronavirus (CoV). Saif and co-workers (Costantini *et al*., 2004) have clearly demonstrated the utility of the pig as a model to understand the mechanisms for 'super-spreaders' and the atypical pneumonia and variable diarrhoea induced by the human CoV responsible for severe acute respiratory syndrome (SARS). The porcine model of SARS consists of utilizing the porcine respiratory CoV (PRCV), a spike deletion mutant of the enteric CoV transmissible gastroenteritis virus (TGEV), which shows striking pathogenetic similarities to the SARS CoV in its primary replication in the lung. Further research is justified to compare known immunological differences and similarities between mice, humans and pigs. Current work by Dawson *et al*. (2008) has revealed that pig immune responses are more similar to human responses than mouse responses for over 80% of the variables compared, and that the mouse immune responses were more similar to human than pig responses is less than 10% of comparisons (Dawson *et al*., 2008). Genomic tools will continue to push existing animal models to evolve and novel models to be developed (Table 17.5).

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Table 17.5 Evolution of animal models generated by genomic tools.

Characteristic features	Traditional view	Current view	Future view
Relevance to disease	Anatomy, physiology, pathology and responses to therapeutics	Disease characteristics and therapies or devices tested	Selected based on specific disease and therapeutic responses
Practical considerations	Dietary and housing requirements, husbandry, genetic uniformity and cost	Restricted to gene-rich species (worms, fruit fly, yeasts, rodents)	Emerging genomic profiles of animals with similar disease phenotypes to humans
Unique features		Emergence of new technologies for gene manipulation; knock-in/knockout; conditional gene activation	Recombineering multi-allelic substitutions; *in vivo* gene expression monitoring; enhanced phenotyping of disease progression; bioinformatics and predictive profiling
Ethical features	Clear laws, regulations and policies	Pain and stress protocol issues	Unknown issues in addition to use of new species for biomedical-regulated animal protocols
Overall characteristics	Practical and economical but relevance to human phenotype may be questioned	Genetically similar but is phenotype similar?	Ideal owing to recapitulating human condition

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