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Review

Animal models for elucidating human disease: confronting cancer and other chronic diseases

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Abstract

The incidence of chronic diseases is increasing, despite advances in clinical medicine. Human diseases are often difficult to decipher because of the complexity of genetics and lifestyles. As such, appropriate biomedical models are essential, since most medical knowledge, treatment regimes, and the development of medical devices that have contributed to clinical advancement are based on robust animal models. Animal models are essential tools for studying gene–gene interactions, gene–environment effects, and for preclinical testing of therapeutic interventions. Given that mice, the most common animal model, frequently do not faithfully recapitulate human diseases, pigs and other large mammals, such as the dog, will continue to serve as important biomedical models. This review discusses animal models used for understanding human diseases and highlights the advantages and disadvantages for each.

Keywords: Animal model, Transgenic animal model, Biotechnology, Chronic disease

The Animal Model Concept

Advances in clinical medicine have increased the human life span to 78.1 years in 2006 and decreased the death rate from chronic diseases, including a 6.4% decline in stroke, a 5.5% decline in heart disease, and a 1.6% decline in cancer incidence between 2005 and 2006 [1]. The incidence of chronic diseases caused by complex genetic and environmental interactions, however, has continued to increase during the past century. As such, appropriate biomedical models are essential since most medical knowledge, treatment regimes, and the development of medical devices that have contributed to clinical advancement are based on robust animal models.

The use of animals to study human physiology and anatomy can be traced back to the second century AD in which Galen, a Greek physician and philosopher, completed research studies on apes and pigs. 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 sixteenth century that his error was initially recognized. In 1865, Claude Bernard, a French physiologist, published *An Introduction to the Study of Experimental Medicine*, which provided a basis for modern research principles. It proposed the use of chemical and physical induction of disease in animals, thus becoming the first published book to advocate creating 'induced animal models' for biomedical research. At the turn of the century came the development and use of animal models for infectious disease and screening, the evaluation of new antibacterial drugs based upon the work of Louis Pasteur in France and Robert Koch in Germany and the introduction of the 'germ theory of disease.' Today, researchers rely on the identification and development of animal models to explore all avenues of human disease. New animal models are continuously being identified and characterized as a result of this need.

Animal models represent important tools to investigate the pathogenesis and for developing treatment strategies for human disease. 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, surgical research and organ transplantation because of the similarity in size, anatomy, and physiology between pigs and humans [2]. Animal models, regardless of species, can be grouped into one of the following five categories: (1) spontaneous models, (2) genetically modified models, (3) induced or experimental models, (4) negative models, and (5) orphan models.

Spontaneous Animal Models

One approach for 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 [3]. However, spontaneous mouse models are not always appropriate; for example, prostate cancer is an area of research in which the mouse is not best suited to study, given the differences in the anatomy and microanatomy of the human and mouse prostate. Researchers have thus utilized the dog as a model because it is the only species other than humans to frequently develop prostate cancer. Moreover, both human and canine prostate cancers are strongly associated with age and have a high propensity for osseous metastases [4]. Libechov minipigs [5], as well as other breeds [6–8], are being employed to study spontaneous melanoma formation. The phenotypic similarity of spontaneous tumour models often extends to similar reactions to treatment in the model animal and the human patient. As such, spontaneous models represent an important approach in the development of treatments for many human diseases.

Despite the significance of spontaneous biomedical models, the long latency of these tumour models makes them impractical for most preclinical studies of tumour progression. Furthermore, in spontaneous animal models there is often only phenotypic similarity and the genetic similarities of disease onset within each species is unknown. The animals may display similar symptoms and the tumours may be physically similar, but the genetic basis of tumour development differs. In the development of a disease the deregulation of one pathway often causes a cascade of events with interactions of multiple genes and gene products, sometimes also using compensatory pathways. These pathways may differ between species and even between individuals or strains of the same species and thus, the resulting tumour is genetically different between species.

Genetically Modified Models

Animal models must recapitulate the genetics of human disease phenotype if they are to effectively serve as a predictive preclinical model of disease treatment. Unlike spontaneous cancer models, it is possible to generate tumours of a defined genetic background in an animal model. Genetically engineered models were created harbouring 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 [9–12]. The introduction of gene-targeting technology in mouse embryonic stem cells allowed for the generation of both oncogene-bearing transgenic mice (gain of function) and tumour suppressor gene knockout mice (loss of function). The resulting mouse strains led to the understanding of the role of individual genes and their mutated counterparts in tumorigenesis.

Conditional strategies have been developed that allow control of gene expression in both tissue- and temporally specific manners. The first of these was based on the use of chemically induced transcription factors, which are responsible for the regulated expression of the target transgene. The most widely used of this type of approach is the tetracycline-dependent regulatory system [13]. A loss of function conditional approach is the somatic deletion of tumour suppressor genes by site-specific and spontaneous recombination using the *Cre*- or *Flp*-mediated recombination [14, 15]. An inherent feature of recombinase-based approaches, however, is a tissue-specific, non-reversible gene switch that does not allow modulation of gene expression in a cell.

Inducible RNA interference (RNAi) systems have emerged as efficient approaches for rapid analysis of gene function that circumvents the limitations of other conditional strategies. The introduction of tightly controlled, stably expressed short hairpin (sh) RNAs can modulate gene expression *in vivo,* is not limited to specific tissue, and is reversible. Inducible shRNA expression using *Cre*-mediated recombination can be utilized to develop reproducible knockdown of gene expression in mice, providing a functional approach for the analysis of gene activity in the mouse [16]. The shRNA system has also been exploited to create a doxycycline-inducible knockdown mouse model of insulin resistance, allowing for temporal control of gene expression that is also reversible [17]. As such, the onset and degree of systemic insulin resistance can be controlled, providing a model for the development of therapeutics targeted at the early phase of Type 2 diabetes onset.

The development of humanized mice has advanced our understanding of human haematopoiesis, innate diseases, cancer biology and regenerative medicine [18]. Humanized mice are immunodeficient mice engrafted with haematopoietic cells or tissues, or mice that transgenically express human genes. Unfortunately, the humanized

mouse and many other genetically modified mouse models do not faithfully mimic the relevant human disease conditions. The ability to generate a genetically modified pig has provided researchers with an enhanced option for mimicking human disease in an animal model. Current research in our lab indicates that the injection of viruses encoding mutated oncogenes and tumour suppressor genes directly into the mammary tissue of a pig is tumorigenic. Pigs subcutaneously injected with viruses encoding mutated forms of *cyclin D1*, *CDK4*, *c-Myc* and *H-Ras* developed an aggressive T-cell lymphoma that spread to most major organs and over half of the lymph nodes in the pig's body (data not published). These studies indicate that genetically modified pigs provide an alternative cancer model that is similar to humans and can be exploited for the study of experimental therapeutics. Work towards creation of a similar model in cloned pigs will generate a tumour model system that employs genetically identical animals with genetically identical tumours for pharmaceutical screening of anticancer therapeutics.

There are several drawbacks to genetically modified models, including the relatively long length of time needed for the animals to develop tumours and the unpredictability associated with tumour frequency and latency of formation. Differing phenotypic expression of genes, depending on the background strain, is a well-known phenomenon among transgenic or knockout lines. Some genes may have a drastic effect, but most often the phenotype is sensitive to modulations by modifier genes [19]. Genetic variability of the tumours formed in many transgenic and knockout models is often exacerbated by alternations in genetic background, with some backgrounds being suppressive and other permissive for tumour development [20].

Induced or Experimental Models

Induced models involve healthy animals in which the condition to be studied is experimentally induced through surgical modifications, genetic modifications, or chemical application. Since 1918, when Yamagiwa and Ichikawa showed that coal tar experimentally applied to rabbit ears caused skin carcinomas, rodent models have long been used to identify carcinogenic potential of chemicals and other agents [21]. More recently, considerable insight has been gained into the strengths and weaknesses of toxicity and carcinogenicity studies in laboratory rats and mice.

In vitro cell transformation is a widely used experimental model in cancer research. In the past, researchers were limited to studying human cancer cell lines derived from human tumour specimens. Malignant transformation in primary cells was first described in birds and rodents by the introduction of either viral or human oncogenes [22, 23]. Murine cell lines have been useful in understanding how genetic alterations drive tumorigenesis. The introduction of certain pairs of oncogenes such as *c-Myc* and *RAS* or the adenovirus *E1A* gene and *RAS* permits the *in vitro* transformation of murine embryo fibroblasts [23, 24]. The ability to rapidly manipulate the expression of oncogenes and tumour suppressor genes in *in vitro* models facilitated the identification of genetic lesions that cooperate to generate a tumour. However, since tumour development differs between mice and humans, mouse models do not accurately recapitulate human tumorigenesis. The neoplastic transformation of human cells requires a different combination of genetic alterations than that of rodent cells [25–28]. In humans, normal somatic cells can be driven to a tumorigenic state via the enforced expression of viral proteins that disrupt the tumour suppressors p53 and Rb, and activate the proto-oncoprotein c-Myc, in conjunction with the mammalian oncogenic protein Ras and the hTERT telomerase catalytic subunit [29, 30]. Only two of these changes are required for rodent cell transformation.

Tumour xenografts, another widely used experimental model, involve growing human cells or human cell lines in immunodeficient mice either ectopically or orthotopically. The advantage of xenografts is that they allow researchers to easily observe the progression of a large number of synchronized tumours, such that initiation of treatment can begin when the tumours have reached an optimal size. Furthermore, xenografts have a high degree of predictability and tumours are rapidly formed. Although these tumours will grow and respond to therapeutics, heterotopic sites are not ideal. Selection of the transplantation site may modulate tumour growth [31, 32] and success of therapeutic invention [33]. In addition, subcutaneous transplanted tumours rarely, if ever, metastasize after implantation [34, 35]. Cells in culture, however, lack the architectural and cellular complexity of *in vivo* tumours, which include inflammatory cells, vasculature and other stromal components. As such, the genetics and histology of the tumours are infrequently representative of the respective tumour and, thus far, these models have not been as predictive of therapeutic success as is needed [36]. Furthermore, the tumours are typically too small for preclinical studies of imaging, hyperthermia, radiation, or photodynamic therapy, thereby limiting xenografts as a suitable model. A large animal model that is representative of the genetic changes associated with human cancers is needed for preclinical studies with the aforementioned devices.

In response to the need for a large animal model, researchers have turned to the pig as an experimentally induced model. Porcine cells can be genetically engineered to recapitulate changes commonly found in human cancers via the inactivation of the tumour suppressors *p53* and *Rb* concomitant with the activation of *c-Myc*, *Ras* and telomerase. When the tumorigenic cells are returned back to the host (isogenic) pig, tumours are formed at the site of injection [37]. The ability to rapidly and reproducibly genetically induce tumours of sizes similar to those treated clinically in a large mammal similar to

humans in many respects will provide a robust cancer model for preclinical studies.

There is often a need for only specific cell types to be transformed when modelling individual cancer types. Somatic cell gene transfer is a method used for conditional oncogenic expression. This technology differs from germ-line modification strategies discussed previously in that the viral vectors are used to transfer genes into a limited number of somatic cells to induce cancer, usually after birth. The use of avian retroviral vectors for gene transfer to mice requires that the mouse cells be genetically modified to express the receptor used by the retrovirus. The most commonly used system utilizes vectors based on the subgroup-A avian leukosis virus, referred to as RCAS vectors [38], and their receptor tv-a [39]. Because tv-a is not normally expressed on mammalian cells, the infection with RCAS is extremely low or non-existent. Mammalian cells must be engineered to express tv-a so they become highly susceptible to infection by RCAS vectors, allowing for specific cell types to be targeted using a tissue-specific promoter [40].

Negative Models

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 [41]. The main application of negative models is to gain insight into the physiological basis of disease resistance. For example, Cui *et al.* established and studied a colony of mice with a unique trait of host resistance to both ascites and solid cancers induced by transplantable cells [42]. This model demonstrates the existence of a host resistance gene that can prevent the growth of advanced cancers and may be one explanation for the existence of individuals who fail to develop cancers, despite prolonged and intense exposure to carcinogens.

Negative animal models also provide researchers with a mechanism to study drug resistance. For example, a common treatment modality for in-transit melanoma of the extremity has been hyperthermic isolated limb perfusion (HILP) with melphalan and more recently, isolated limb infusion (ILI) with melphalan. Research in the area has primarily focused on maximizing drug delivery through a better understanding of pharmacokinetics while maintaining acceptable levels of toxicity. Resistance mechanisms to melphalan are studied using animal models of HILP and ILI in an effort to improve the clinical response in patients with in-transit melanoma of the extremity [43].

While negative models are not as commonly used as spontaneous and genetically modified models for cancer research, they still have the potential to impact the development of cancer therapeutics. Further research of the model described above may identify the cellular and genetic machinery necessary to reject a fully developed malignancy and thus, may suggest a potentially feasible strategy for treatment of advanced cancers that could be translatable into human patients.

Orphan Models

There are functional disorders present in non-human species but have not yet been described in humans. Examples include Marek's disease, papillomatosis, bovine spongiform encephalopathy (BSE) and feline leukaemia (FeLV). Often a similar disease will be identified in humans that were previously described in animals. These animals become orphan models for that particular disease. Feline leukaemia is 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 human diseases are characterized by defects of multiple cellular immunologic parameters. Both Hodgkin's and non-Hodgkin's lymphoma patients have a tendency to develop opportunistic infections because of a compromised immune system resulting from the cancer. Studies on FeLV could provide insight into the molecular and cellular events of lymphoma-related immunosuppression. This model is applicable to *in vitro* evaluation of pharmacologic agents for potential reversal of immunologic deficits associated with lymphoma as well as *in vivo* evaluation of the effects of chemical and radiologic treatment modalities for lymphoma [44].

Extrapolation from Animals to Humans

The selection of an animal model depends on the 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 of a human disease. For diseases such as cancer, there is a wide range of well-described models available, both induced and spontaneous in a variety of species (Table 1). The key factor in using animal models for studying disease is the extrapolatability of the results to 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 [41]. 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 to 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. While many animals are more or less similar to humans in regard to biological characteristics, there are prominent differences in body size between species,

Table 1 Advantages and disadvantages of animal models of cancer

Type of model	Advantages	Disadvantages	Example
Spontaneous	Similar disease phenotype	Long latency Not genetically defined	Canine prostate cancer
Genetically modified	Defined genetic background of tumours	Unknown tumour latency and frequency Phenotypic expression of genes can differ Genetic variability associated with tumours Host does not require immunosuppression	Porcine malignant lymphoma
Induced or experimental	Ability to rapidly manipulate gene expression	Neoplastic transformation requires different genes depending on species	*In vitro* porcine cell transformation
	Rapid tumour formation	Do not represent effects of tumour microenvironment	Murine xenografts
	Free choice of species	Host must be immunosuppressed	
Orphan	Useful for evaluation of chemical/ radiological treatments	Do not always faithfully mimic human disease	Feline leukaemia

which affects their appropriateness as a model for certain experiments. The pig, for instance, shares anatomic and physiologic characteristics with humans that make them a unique and viable model for biomedical research [45]. The cardiovascular anatomy, physiology and 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 [46]. Swine are also a standard model for skin and plastic 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 [46]. The anatomic and physiological similarities between humans and pigs have made it the choice biomedical model for a number of diseases. Moreover, the sequence homology of pigs with humans is approximately 60%, as compared with only 40% for rodents due to a higher rate of nucleotide substitution [47].

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 concordance of the toxicity of pharmaceuticals observed in humans with that observed in experimental animal models [48]. 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 if there is an alternative species that might be more appropriate for testing a specific compound. Recent advances in genomics and comparative mapping techniques have demonstrated that the genomic sequence homology between humans and pigs is high [2] 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 humans than, for example, mice [49, 50]. As such, toxicology studies carried out in pigs may be more appropriate and the data collected may be more appropriate for determining if a compound is going to be toxic to humans.

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 due to genetic and environmental influences. An animal model of cancer should ideally undergo tumour development and progression in a similar fashion to humans. As with humans, the incidence of cancer in pigs is rare, with a prevalence of the childhood cancer Wilm's tumours [51] in young pigs and a broader spectrum of cancers in adults [52]. The parallels

Table 2 Criteria of an ideal animal tumour model

Criterion	Description
Biological	Multistage Tumour initiation takes place in single cell Tumour progression is associated with specific changes in a single cell Tumour histology and pathology are similar to equivalent human tumour
Genetic	Multiple mutations in specific genes Gross chromosomal changes in regions orthologous to those altered in human disease Tumours have specific alterations in one or more pathways known to be involved in human cancer Gene expression profiles similar to human tissues
Environmental effects	Reflects changes resulting from exposure to factors known to initiate tumour development in humans: Mutagens (chemical, UV, radiation) Tumour promoters Dietary factors Hormones
Therapeutic	Suitable for the testing of new therapies directed at specific targets Predicative of therapeutic results in humans

in cancer biology between human and pigs are conserved at a molecular level. For example, in both species telomerase is suppressed in a number of tissues but reactivated during cancer [7, 53].

Model Validation

The extent to which it is possible to extrapolate from animals to humans, and, therefore, the value of information derived from an animal model, depends to a large extent on the validity of the model. Effective biomedical models should rate highly against the following criteria: (1) fidelity, (2) predictive validity, (3) homology, (4) isomorphy, (5) face validity and (6) construct validity.

The extent a biological structure in an animal resembles that of a human has an impact on the effectiveness of modelling a particular disease in any given species has been termed *fidelity*. A high-fidelity animal model gives a highly relevant biological closeness to the human structure, thereby providing an obvious advantage. What is often more important, however, is the discriminating ability of the model, or its *predictive validity*. For example, when using an animal model to assess the carcinogenicity of a substance, it is critical that the animal responds in a manner that is predictive of the human response to this substance. Thus, the similarity between humans and model species with respect to relevant biological mechanism is often more important than the fidelity of the model [54]. These criteria are often closely intertwined, and high-fidelity models offer the best opportunity to study a particular biological function.

An animal model may be considered *homologous* if the symptoms shown by the animal and the course of the condition are identical to those in humans [55]. Models fulfilling these requirements are relatively few, but an example is animal models of depression in which 'learned helplessness' has been demonstrated [56]. An animal model is considered *isomorphic* if the animal symptoms are similar, but the cause of the symptoms differs between human and model. However, most models are neither homologous nor isomorphic but are termed *partial* because they do not mimic the entire human disease but may be used to study certain aspects or treatments of the human disease [57].

The degree to which there is a similar phenotypic display between the disease in the animal and the corresponding disease humans is termed *face validity*. For example, it could be argued that the demonstration of drug effects in an animal model for depression after a period of chronic administration is important for establishing its face validity, but is not relevant to the model's predictivity and thus, its ability to serve as a screening test for treatments for the modelled disease [56].

Finally, the degree to which there is a similar genetic display between the disease in the animal and the corresponding disease in humans is termed *construct validity*. As an example of high construct validity, research was performed on three candidate dopaminergic genes (DRD2, DRD4 and DAT-1) that were sequenced in spontaneous hypertensive (SHR) and Wistar Kyoto (WKY) rats. There were no profound differences identified in DRD2 or DRD4 genes, but several variations were found in the DAT-1 gene that were of significance because several gene families associated with attention-deficit hyperactive disorder (ADHD) showed linkage to DAT-1 [58]. The validity of using WKY as a control for SHR was strengthened because the behavioural characteristics are similar to those of other rat strains.

Building an Animal Model of Cancer: A Porcine Case Study

Animal models are selected because of the pertinent attributes of the disease mechanisms that are implicated in the genesis and progression of cancer. This includes the study of tumour biology and the mechanisms of

carcinogenesis, the investigation of infectious agents related to cancer development, and in the preclinical assessment of novel anti-cancer therapies and diagnostics. The ideal cancer model faithfully recapitulates the progression of tumorigenesis from initiation to metastasis. The model must demonstrate high penetrance but low tumour number, so the animals do not die prematurely from overload by multiple benign tumours [59]. Ideally, tumours develop with a short latency, allowing for tumour progression and analysis within a short period of time. Table 2 outlines the specific criteria characterizing an ideal tumour model. To date, no single model recapitulates every single aspect of cancer as observed in humans because human cancers are very heterogeneous, even within a particular type such as leukaemia. Existing models do, however, accurately mimic one or more important features of a particular human cancer type (Table 2). Many animal models of cancer recapitulate one or more stages of tumour progression.

Conclusions

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 Initiative 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. As genomic and bioinformatic technologies continue to advance, our knowledge of the appropriate animal model will increase, refine our choice of models and create more applicable models. New, more precise models for the development of therapeutics can be created. Animal models are essential tools for studying gene–gene interactions, gene–environment effects and for preclinical testing of therapeutic interventions. Given that mice, the most common animal model, do not faithfully recapitulate human disease, swine will continue to serve as an important biomedical model. Continued advancements in genomic technology will help ensure the continued development of the pig as a human disease model.

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