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Review

Advances in Swine Biomedical Model Genomics

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This review is a short update on the diversity of swine biomedical models and the importance of genomics in their continued development. The swine has been used as a major mammalian model for human studies because of the similarity in size and physiology, and in organ development and disease progression. The pig model allows for deliberately timed studies, imaging of internal vessels and organs using standard human technologies, and collection of repeated peripheral samples and, at kill, detailed mucosal tissues. The ability to use pigs from the same litter, or cloned or transgenic pigs, facilitates comparative analyses and genetic mapping. The availability of numerous well defined cell lines, representing a broad range of tissues, further facilitates testing of gene expression, drug susceptibility, etc. Thus the pig is an excellent biomedical model for humans. For genomic applications it is an asset that the pig genome has high sequence and chromosome structure homology with humans. With the swine genome sequence now well advanced there are improving genetic and proteomic tools for these comparative analyses. The review will discuss some of the genomic approaches used to probe these models. The review will highlight genomic studies of melanoma and of infectious disease resistance, discussing issues to consider in designing such studies. It will end with a short discussion of the potential for genomic approaches to develop new alternatives for control of the most economically important disease of pigs, porcine reproductive and respiratory syndrome (PRRS), and the potential for applying knowledge gained with this virus for human viral infectious disease studies.

Key words: swine, humans, genomics, gene expression, biomedical model

1. Introduction

Swine have served as an important biomedical model for humans for decades; previous authors have summarized such models in more detail [1-5]. This manuscript is a short update of the diversity of swine biomedical models. Limitations on reference citations for this review has meant that only the most recent will be cited to cover the latest developments and the broadest range of current pig models. The review will discuss some of the genomic approaches used to probe these models. Advances using these genomic tools are described in the other reviews in this issue [6, 7].

Generally speaking, studying animal models of human disease helps scientists understand the mechanisms involved in the disease pathogenesis and thus provides tools for development of gene therapy to cure the disease/condition in humans. To date, the humanized mouse has been widely used to advance our understanding of human hematopoiesis, innate and adaptive immunity, autoimmunity, infectious diseases, cancer biology and regenerative medicine [8]. Unfortunately, the humanized mouse and many mouse disease models often do not faithfully mimic the relevant human conditions. Therefore, better animal models are needed. An example is the well developed swine atherosclerosis model which has facilitated analyses of disease progression and pathogenesis and testing of new therapeutics [9, 10].

This review will probe, as detailed examples, genomic studies of melanoma and of infectious disease resistance, highlighting issues to consider in designing such genomic studies. It will end with a short discussion of the potential for genomic approaches to develop new alternatives for control of viral infectious diseases, targeting porcine reproductive and respiratory syndrome virus (PRRSV), and the potential for applying knowledge gained with this virus for human infectious disease studies.

2. Advantage of the Swine as a Biomedical Model

As outlined in Table 1 the swine has been used as a major mammalian model for human biology [11]. The similarity in size, particularly for miniature pigs, and physiology, and in organ development and disease progression make the swine an ideal model. The ability to deliberately time studies, image internal vessels and organs using standard human technologies, and collect repeated peripheral samples and, at kill, detailed tissue samples, has meant that the pig is an excellent biomedical model for humans. The ability to use pigs from the same litter, or cloned or transgenic pigs, facilitates genetic mapping. Availability of numerous well defined cell lines, representing a broad range of tissues, will facilitate detailed testing of gene expression, drug susceptibility, etc. For genomics it is an asset that the pig genome has high sequence and

chromosome structure homology with humans. With the swine genome sequence now well advanced there are increasingly improving genetic and proteomic tools for pigs.

Table 1. Advantages of Swine as a Biomedical Model

- Human size – particularly miniature pigs
- Physiology similar to humans
- Large litter sizes
- Cloning and transgenic technology well-advanced
- Numerous well defined cell lines
- Similar disease progression
  - metabolic , e.g. obesity and heart disease
  - infectious diseases – numerous organisms cause infections across species
- Ability to deliberately time studies and collect repeated and, at kill, detailed tissue samples
- High sequence and chromosome structure homology with humans
- Improving genomic and proteomic tools

3. Swine Biomedical Models

Swine have served as an important biomedical model for decades. Table 2 highlights some of the broad array of biomedical topics now addressed using swine models. Some of these studies already employ genomics approaches, such as the heart, transplantation and melanoma models; others are still in the early stages of affirming swine physiological parameters and utility as a human biomedical model. Each model will be impacted by the availability of the functional genomic tools and swine genome sequence and maps outlined in other reviews in this issue [6, 7].

Some of the best examples of the impact of swine as a biomedical model are found with atherosclerosis and diabetes, diseases that are increasingly important today as the US faces major problems with obesity [9, 10]. Research is underway in pigs to determine the role of genetic background and to identify nutritional, exercise, and drug approaches which will alleviate disease progression and prevent pathology. Advances in strategies to treat myocardial infarction are being pursued with cellular myoblast repair strategies [12] and tissue engineering of blood vessels [13]. Testing of emergency room treatment options, such as directed cardiopulmonary resuscitation (CPR) and ventricular fibrillation or cardiopulmonary bypass, which are difficult to assess in humans, are readily testable with pigs. Genomics will provide in depth analytic tools to probe these pig models in detail.

All aspects of reproductive function have been studied in the pig, from the basics of maternal-fetal interactions [14], embryo development [15-17] and the impact of transgenesis [18, 19] to sperm function and quality [20, 21]. Basic sperm biology, such as chromosome positioning during spermatogenesis [22], as well as semen transmission of infectious disease [23, 24] are under active investigation (Table 2). Major transcriptional genomic and mapping efforts are underway in the pig model [14, 18] and should reveal important pathways regulating reproductive function.

Table 2. Swine Biomedical Models

Model	*Current Ref.*
Heart physiology	
Stent design, tissue engineering of blood vessels	[25, 26]
Atherosclerosis	[9, 10]
Myocardial infarction	[27, 28]
Ex vivo heart model	[29]
Emergency procedures	[30, 31]
Reproductive function	
Maternal-fetal interactions	[14]
Embryo development	[15-17]
Sperm	[20, 21]
Transplantation	
Cell and organ transplants	[32, 33]
Xenotransplantation	[5, 34, 35]
Drug therapies and biotherapeutics	
Skin physiology	
Percutaneous permeation	[36, 37]
Contact dermatitis	[38]
Skin equivalent culture model	[39]
Melanoma	[40,41]
Brain	
Stroke - focal cerebral ischemia	[42]
AIDS dementia - Multinucleated giant cell formation	[43]
Drug binding sites and interactions	[44]
Gut physiology and Nutrition	
Gut structure and intestinal metabolism	[45, 46]
Obesity	[47]
Probiotics and gut physiology	[48, 49]
Biologic and immunological basis of food allergies	[50, 51]
Biomechanical models	
Response to injury	[52]
Imaging techniques	[53, 54]
Bone density analyses - Osteoporosis	[55]
Tissue engineering	
Cartilage repair - chondrocytes	[56]
Spinal fusion	[57]
Organ specific gene delivery	[58]
Lens capsule epithelial cells for cataract repairs	[59, 60]
Polymer scaffolds	[61, 62]
Tooth development - dental enamel	[63]
Respiratory function	
Neonatal respiratory distress	[64]
Thoracic artificial lung	[65]
Disease models and therapies; Asthma	[66,67]
Infectious disease models	
Therapeutics: Vaccines, Biotherapeutics, Drug therapies	[68, 69]
Developmental Interactions	[70, 71]
Mucosal tissue responses	[72-75]
Genomics of host responses	[76]

Because of the size and physiologic similarity of pigs to humans, the pig has become a model of choice for tissue engineering and imaging studies. A range of imaging techniques has been developed with pigs as an early pre-human validation model. For example, sentinel node detection is increasingly important for cancer therapeutics; the pig model has informed techniques for laparoscopic colon visualization and resection procedures [53, 54]. Tissue engineering using polymer scaffolds [61] have targeted areas as diverse as alternatives for knee meniscus cartilage and artificial bladder construction [62].

Swine skin studies have been very important, the swine melanoma model has been particularly informative. Targeted studies analyzing percutaneous permeation with different chemicals will influence international biodefense efforts as well as responses to biological toxins [77]. Gut physiology and intestinal development following probiotics have been pursued in pigs both as a means of decreasing antibiotic usage in pig feed as well as an important human model. Assessments have focused on probiotic strain selection,

timing and dosing and the effects of these particularly on neonatal gut physiology.

4. Genomics and Melanoma: A case study

The swine melanoma model is a well established spontaneous melanoma model and one of the best developed for genomic approaches. The Sinclair melanoma and Melanoma-bearing Libechov Minipig (MeLiM) have been studied in detail using immune analyses, focusing on the role of tumor infiltrating lymphocytes, the potential effects of natural killer (NK) and γδ T cells in tumor regression. Imaging and sentinel lymph node (SLN) mapping will enhance these studies [54]. Comparative studies of normal melanocytes with localized tumor cells should reveal tumor specific regulatory pathways [78]; indeed laser capture microdissection studies revealed loss of the 13q36-49 chromosomal region in nodular melanoma cells [79].

Early mapping studies in the Sinclair swine melanoma model determined that a single dose of a specific "B" swine leukocyte antigen (SLA) haplotype on SSC7 was required for tumor initiator [80]; complex segregation analysis identified a second locus [81]. These were followed by more detailed QTL studies using the MeLiM model which identified numerous melanoma candidate loci [40]; interestingly human candidate genes CDK4 and BRAF were not susceptibility genes in this model. Zhi-Qiang [41] continued these studies identifying QTL for the synthetic trait, development of melanoma on SSC1, 2, 13, 15 and 17. Their detailed phenotyping of 331 pigs revealed highly significant QTLs ($p < 0.001/p < 0.05$ respectively, chromosome-/genome-wide levels) for precise disease traits. These included SSC10 42.0 cM for ulceration; SSC12 95.6 cM for presence of melanoma at birth; SSC13 81.0 cM for lesion type; SSC16 45.3 cM and SSC17 44.8 cM for number of aggressive melanomas; and the SSC1 MeLiM MC1R*2 allele for black coat color predisposing to melanoma. As outlined in Table 3 these studies affirmed that more exact mapping of complex traits such as tumor growth and regression are improved when very detailed phenotypic information is collected on a large group of animals.

Table 3. Lessons learned from Swine Melanoma Studies

- Well established and characterized models
  - Sinclair melanoma
  - Melanoma-bearing Libechov Minipig (MeLiM)
- Large numbers of affected individuals for mapping studies
- Detailed phenotypic information
  - Comparative analyses of normal versus tumor tissues
  - Assessment of factors determining development of pathology
  - Well defined tumor regression
- Access to cancerous tissues
  - Analyses of specific tumor subsets
  - Cell migration and tumor infiltrating lymphocyte analyses
  - Definition of regulatory pathways

5. Swine as infectious disease models

As scientists evaluate methods to prevent infectious diseases and test new therapies and vaccines the pig is an ideal choice. Approaches include probing mucosal tissue responses in respiratory [72], reproductive [23, 24], neurological [73], and intestinal infections [74, 75], testing biotherapeutics and drug therapies, probing the effects of disease on development [70, 71] and testing therapies for specific ailments, e.g., asthma [66, 67]. Major efforts to determine the genomics of host responses using transcription and proteomic analyses are in early stages [82] Transgenic and mapping approaches [76, 83] will help to affirm specific gene and allele involvement. Table 4 outlines some of the major issues to consider as one attempts to use swine models for biomedical studies.

Table 4. Utilizing genomics for Swine Models - Issues to Consider

- Genotyping
  - Population Design
  - Mapping or gene/protein expression
  - Increasing number of SNPs available for swine
- Phenotyping
  - Extensiveness of phenotype improves ability to reveal full details of genetic control
  - Importance to sample local tissues, not just peripheral blood or mucosal secretions
  - Additive information provided by in vitro cellular systems
  - Impact of imaging techniques on expanding phenotype
- Candidate genes versus hypothesis independent analyses
  - Experimental effort required to consider numerous candidate genes
  - Potential for unbiased arrays/ comparative maps
  - Datasets – Current limits on number/complexity of samples to compare phenotype with genotype

6. Pig anti-Viral Responses: the response to Porcine Reproductive and Respiratory Syndrome virus

Regulation of immune responses and genetic resistance to infectious viral diseases is an area of concern for human and swine. Porcine Reproductive and Respiratory Syndrome (PRRS) is caused by the PRRS virus, an enveloped, single-stranded positive-sense RNA virus. When present in a herd, PRRSV causes increased abortions, stillbirths, mummies and chronic respiratory problems in pigs resulting in >$560 million losses in the US each year [84]. As an RNA virus with an evolving genome, PRRSV is particularly problematic due to slow development of protective immunity to homologous challenge and lack of protection against heterologous virus challenge. Thus it is a major target for swine research; information gained from swine studies will inform human infectious disease studies, particularly for analyses of viral persistence and of factors relevant to prevention of congenital and seminal transmission pathways.

Major efforts are underway to identify factors regulating PRRSV immunity, persistence and transmission. Tests involve probing local mucosal anti-viral responses (Petry et al., submitted). Detailed cellular analyses have assessed gene and protein expression. As with human disease studies analyses of cultured cells, e.g., infected MARC cells [85], swine macrophages [86, 87], or samples from infected pigs [88], have expanded our knowledge of the impact of timing and level of viral infection on gene expression and pathway involvement. Future work will determine whether RNAi approaches will be effective. More de-

tailed gene expression analyses are underway using long oligo and Affymetrix arrays, testing both pooled and individual animal samples, as well as mucosal samples at death. An important issue is the timing of mucosal sample collection. Results can be disappointing if mucosal samples are collected only after viral levels begin to resolve. The peak of anti-viral immunity (and relevant gene expression) may be much earlier; however, that may be at a time when the actual viral levels may be difficult to evaluate and thus complicate comparative analyses. Therefore, it is important to affirm preliminary results with hypothesis driven repeated analyses and with translation of gene expression and array results into protein expression data.

Genetic variation does exist in resistance/susceptibility to viral infections and has been proven for swine resistance/susceptibility to PRRS [87, 89]. Although to date there is limited knowledge of host genes determining PRRSV resistance; some candidate genes have been identified (Petry et al., submitted). More detailed studies are required to determine whether naturally disease resistant pigs can be identified and why do [some] pigs stay healthy even with PRRS? What set of factors (detailed phenotype) truly correlate with lower PRRSV burden? What is the potential for sampling peripheral blood cells, serum or saliva for preinfection predictive studies of genetically determined virus resistance phenotype?

An international PRRS Genomics Consortium, of university, government, and company based scientists, has been established to assess host genetics of PRRS resistance/susceptibility. The goal is to develop a large, publicly available disease sample and dataset from thousands of pigs from relevant commercial lines infected with PRRSV and from which a detailed phenotype have been collected. Access to samples will be dependent on data sharing. The end user performs his/her analysis on the appropriate sample and returns the data to the consortium. It is hoped that the data generated by the Consortium will verify the genetic variation in pigs responding to PRRSV infection, will reveal factors influencing health, survivability and growth, and will identify the relative importance of different phenotypes, and their heritability, in predicting responses to PRRSV infection. Overall this data should enable breeders to produce healthier pigs with improved resistance to PRRSV and help animal health companies to develop improved vaccines and alternative anti-PRRSV therapeutics. This data should help identify new critical control factors in human responses to viral infections.

Conflict of interest

The author declares that no conflict of interest exists.

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