"The NSRRC and Genetic Engineering of Swine"

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University of Missouri-Columbia
Swine are being used extensively as biomedical models of human health (Comparative Medicine)

- Xenotransplantation, Organ Transplantation
- Ophthalmology
- Cystic Fibrosis
- Cardiovascular disease/Atherosclerosis
- Diabetes
- Muscular Dystrophy, Spinal Muscular Atrophy
- Pharmaceutical Production
- Cancer
- Cutaneous pharmacology/Wound repair/Dermatology
- Toxicology
- Lipoprotein metabolism
Things that Count in a Biomedical Model (1 of 2):

• Availability Counts

• Phenotype Counts
  – Mice don’t always have the same phenotype as humans (Cystic Fibrosis)

• Physiology Counts
  – There are metabolic and hemodynamic differences between rodents and humans (Cardiovascular Disease)
  – Pig mammary gland can properly post-translationally modify the Vitamin K-dependent proteins of hemostasis (Pharmaceutical Production)
Things that Count in a Biomedical Model (2 of 2):

- **Size Counts**
  - Difficult to translate therapeutics (Spinal Muscular Atrophy)
  - Difficult to take blood flow measurements on rodents (Cardiovascular Studies)
  - Difficult to acquire enough tissue for proteomics from rodents
  - Organ size of pigs is similar to humans (Xenotransplantation)
  - Rodents are difficult on which to practice techniques (Osteogenesis Imperfecta)

- **Genome Counts**
  - Ability to genetically alter the genome
  - Availability of inbred strains
  - Sequenced genome
  - Genome similarity to humans
    - Phylogenetically the pig is 3X closer to the human than the mouse to human
    - Synteny of pig is closer to human than the mouse is to human

- **Acceptability as a Preclinical Model Counts**
At the University of Missouri, a genetic resource for the biomedical community 

OD011140, ORIP, NIAID, NHLBI
NSRRC Function/Operation

- Importation/Rederivation/Distribution/Repository
- Health Monitoring
- New Model Creation
  - PI-driven
  - Steering Committee (bi-monthly)
- Advisory Board
- Advertisement
- Workshops/Training
Challenges/Opportunities

• Challenges
  – Gestation length (4 months)
  – Puberty (7-9 months)
  – Few inbred strains

• Opportunities
  – Large animal with physiologically relevant phenotype
Swine Models

• Some naturally occurring models are useful:
  – Ossabaw Island Pigs- Type II diabetes
  – Apo B- Hypercholesterolemic
  – Etc.

• Additional genetic modification can create models that are otherwise unavailable.
Methods to Make Genetically Modified Pigs

- Pronuclear Injection.
- Sperm-Mediated Transfection.
- Oocyte Transduction.
- Genetic Modification followed by Nuclear Transfer (Somatic Cell Nuclear Transfer (SCNT)).

Future-
- Germ Cell Transplantation?
- Stem Cell Technology (Chimeras or SCNT)?
Enucleation

Synchronize?

Nuclear Transfer

Development

Fusion or Injection and Activation

Transfect or Transduce and Select
Swine Gene Modifications @ MU:

- Xenotransplantation

**ORGAN TRANSPLANTATION**

To solve a deficiency of human organs for transplantation, scientists want to use organs from other species. MU researchers are working to prevent rejection of the donated organ by the body.

<table>
<thead>
<tr>
<th>NORMAL</th>
<th>MANIPULATED</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <strong>Normal DNA</strong> contains instructions for sugar molecules on the surface of each cell in the transplanted organ.</td>
<td>1. Researchers alter the DNA to disrupt the creation of sugar molecules on the surface of the cells.</td>
</tr>
<tr>
<td>2. <strong>Human anti-bodies</strong> attach to the <strong>sugar molecules</strong>.</td>
<td>2. <strong>Without the sugar molecules anti-bodies</strong> cannot connect to the cell.</td>
</tr>
<tr>
<td>3. The anti-bodies kill cells in the organ causing hyper-acute rejection.</td>
<td>3. <strong>So the organ is not rejected.</strong></td>
</tr>
</tbody>
</table>

Source: Randy Prather, MU professor of animal science

CHRIS ROYER/Missourian
Swine Gene Modifications @ MU:

• Xenotransplantation
  – Hyperacute Rejection
    • α1-3 Galactosyltransferase (GGTA1) KO: (Lai et al ’02 Science)- Immerge Biotherapeutics US Patent # 7,547,816
Swine Gene Modifications @ MU:

- **Xenotransplantation**
  - Hyperacute Rejection
    - **GGTA1 KO**: (Lai et al ’02 Science)
    - **Cytidine Monophospho-N-Acetylneuraminic Acid Hydroxylase (CMAH) KO**: (Lee et al unpublished)- KonKuk University, Korea

Born April 4, ‘12
Swine Gene Modifications @ MU:

- Xenotransplantation
  - Hyperacute Rejection
    - **GGTA1 KO**: (Lai et al ’02 Science)
    - **CMAH KO**: (Lee et al unpublished)
    - **hDAF on the GGTA1 KO background** (Lai et al unpublished)

E.g. **DAFney**: Born July ’04. hDAF and GGTA1 KO. Base genetics from Imutran Ltd.
Swine Gene Modifications @ MU:

- Xenotransplantation
  - Hyperacute Rejection
    - **GGTA1 KO**: (Lai et al ’02 Science)
    - **CMAH KO**: (Lee et al unpublished)
    - **hDAF on the GGTA1 KO background**: (Lai et al unpublished)
    - **hDAF into GGTA1****: (Beaton et al ‘11)

Born December 13, ’10
Alternate use of only 2 phage integrase systems allows for unending insertion into the same locus

Mendel’s Cruel Laws
Mate Heterozygous animal

<table>
<thead>
<tr>
<th>Genes</th>
<th>Usable Pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1/4</td>
</tr>
<tr>
<td>2</td>
<td>1/16</td>
</tr>
<tr>
<td>3</td>
<td>1/64</td>
</tr>
<tr>
<td>4</td>
<td>1/256</td>
</tr>
<tr>
<td>5</td>
<td>1/1,024</td>
</tr>
</tbody>
</table>

Beaton et al, unpublished
hCD55 Expression in heart, liver, lung, kidney and pancreas in control and hDAF into GGTA1.
Swine Gene Modifications @ MU:

• Xenotransplantation
  – Hyperacute Rejection
    • **GGTA1 KO**: (Lai et al ’02 Science)
    • **CMAH KO**: (Lee et al unpublished)
    • **hDAF on the GGTA1 KO background**: (Lai et al unpublished)
    • **hDAF into GGTA1**: (Beaton et al ‘11)
  – Post-Hyperacute Rejection (Acute Vascular Rejection)
  – Cell Mediated Rejection
  – Non-Vascular Rejection (Neurodegenerative Disorders)
  – Porcine Endogenous Retroviruses
Xenotransplantation Combination

• **GGTA1 KO with the following transgenes to deal with both Hyper- and Post Hyper-Acute Rejection:**
  
  - **CD55**: decay accelerating factor; inhibits complement system
  - **CD59**: complement regulatory protein
  - **ENTPD1**: aka CD39, ectonucleoside triphosphate diphosphohydrolase 1; inhibits platelet aggregation
  - **THBD**: Thrombomodulin; anticoagulant

Dr. Simon Robson, Beth Israel Deaconess Medical Center.
Dr. Peter Cowan, St. Vincent’s Hospital, Australia.

Born March 28, ‘11
Swine Gene Modifications @ MU:

- Models of Human Diseases
  - Retinitis Pigmentosa

Born May 26, ‘08

53-1 had a decrease in both a functional rod and core response at 3, 7 and 9 months of age.
• Mendelian inheritance
  9/20 offspring

• 80% of the colonies represented P23H RHO
63-2 has a single layer of cone nuclei in the outer nuclear layer (ONL) and a reduction of nuclei in the inner nuclear layer (INL).

51-1 and 63-1 have less photoreceptor degeneration although outer segments are shorter and the ONL thickness is reduced.

Ross et al ‘12
Cystic Fibrosis

- Michael J. Welsh, et al. University of Iowa
  - PPG P01 NHLBI
  - Subcontract to the University of Missouri-Prather

- Caused by mutation of a single gene (cystic fibrosis transmembrane conductance regulator: CFTR).
- Pig lungs share many anatomical, histological, biochemical and physiologic features with human lungs. Pigs are an excellent model for abnormalities in disease, including pulmonary infections.
- Pig airway epithelia and submucosal gland function resemble humans and is dependent upon CFTR.
- Goal was to make a CFTR KO (exon 10) and a ΔF508 pig.
Cystic Fibrosis

- Defective Chloride ion transport:
  - Intestine
  - Gallbladder
  - Pancreas
  - Bile duct
  - Liver
  - Male genital tract
  - Lung
Cystic Fibrosis

• Autosomal recessive
  – Carrier rate of 5% in Caucasians
  – Named: *cystic fibrosis transmembrane conductance regulator* (CFTR)
  – 70% of the people with CF have a ΔF508
Cystic Fibrosis

• CFTR KO Mice don’t develop the characteristic abnormalities.
  – Meconium ileus
  – Pancreatic destruction
  – Focal biliary cirrhosis
  – Lung disease
  – Occluded/absent vas deferens

• Thus they are not a good model for studying CF in humans.
Immuno-cytochemistry of CFTR in airway epithelia (top) and ileum (bottom). Figures are differential interference contrast with staining for ZO-1 (a component of tight junctions, red), CFTR (green), and nuclei (DAPI, blue). Bars, 10 μm.

Rogers et al., '08 Science
Gross appearance of gastrointestinal tract. Piglets were fed colostrum and milk-replacer for 30-40 h and then euthanized. Stomach (black *), small intestine (arrowheads), pancreas (white arrow), rectum (white *), and spiral colon (black arrow). Of 16 CFTR–/– piglets, the obstruction occurred in small intestine in 7 and spiral colon in 9 piglets.

Rogers et al., '08 Science
Microscopic appearance of the colon. H&E stain. Bars, 1 mm. Images are representative of severe meconium ileus occurring in 16 of 16 CFTR−/− piglets.

Rogers et al., ‘08 Science
Corrective Surgery

- CFTR\(^{-/-}\) and ΔF508 CFTR piglets had an ileostomy or cecostomy.
- Piglets had pancreatic insufficiency.
- At 10 weeks of age – had something similar to distal intestinal obstruction syndrome.
- This was corrected.
Matching mucus traits. (A and B) These airways, obtained during a lung transplantation performed on a 15-year-old CF patient, show two kinds of mucus obstructions observed in the removed CF lungs: a more common form of purulent, ivory, semi-liquid mucus [see arrows in (A)] and a less common form of translucent, resilient, elastic mucus, shown here being stretched and pulled from a bronchus [(A) and (B)]. Compare with (C), in which translucent mucus is being pulled from the airway of a CF pig described in Stoltz et al. and shown in their Fig. 3B, right panel. J.J.Wine 2010 Science Translational Medicine April 29.
Conclusions

• In contrast to mice, Pigs develop the characteristic abnormalities associated with CF (Rogers et al ‘08)
  – Defective chloride ion transport
  – Meconium ileus
  – Partial pancreatic destruction
  – Focal biliary cirrhosis
  – Congealed bile & duct blockage
  – Lung Disease
  – Develop a blocked vas deferens (Pierucci-Alves et al ‘11)

• Bacterial infection comes before the inflammation (Stoltz et al ‘10)

• Reduced IGF-1 at birth may explain slightly smaller statue of humans with CF (Rogan et al ‘10)

• $K18$-$rtTA$, $K18$-$tTS$, $TRE$-$CFTR^+$ is being tested to rescue the gut phenotype of the $CFTR$ $\Delta F508/\Delta F508$ (unpub.)
Swine Gene Modifications @ MU:

• Models of Human Diseases
  – Retinitis Pigmentosa
    • *Rhodopsin – P23H NIH SLA* background: (Ross et al ’12, NSRRC)
  – Cystic Fibrosis
    • *CFTR -shRNA* approach: (Unpublished- NSRRC S. Fahrenkrug, UM) – Gene was silenced.
    • *CFTR - KO*: (Rogers et al ’08 J. Clinic. Invest., Science) M. Welsh, U. Iowa
    • *CFTR- ΔF508*: (Rogers et al ’08 J. Clinic. Invest.) M. Welsh, U. Iowa
    • *Intestinal Tet-On CFTR on the CFTR ΔF508 or CFTR -/- pigs*: M. Welsh, U. Iowa
  – Cardiovascular Disease
    • *Endothelial cell-specific reduction of nitric oxide*: (Tie2-Catalase; Whyte et al ’11)
    • *Endothelial cell-specific increase of nitric oxide*: (Tie2-eNOS; Samuel et al unpublished)
Swine Gene Modifications @ MU:

- Models of Human Diseases
  - Muscular Dystrophy
    - **Becker Muscular Dystrophy (DMD+/−) TALEN-mediated (deletion of exon 46)**: Jason Ross, Iowa State. born Feb 4, ’12.
  - Diabetes
  - Spinal Muscular Atrophy
    - **SMN+/−**: (Lorson et al ‘11)
    - **SMN+/−; hSMN2+**: (Lorson et al Unpublished). Born May 23, ‘11
  - Cancer
      LoxP-Stop-LoxP, G12D KRAS, R167H p53
Swine Gene Modifications @ MU:

- Pharmaceuticals

FVIII Born March 10, ’08

FIX Born September 28, ‘08
Swine Gene Modifications @ MU:

- Cell Tracking/Tools
  - **eGFP**: (Cabot et al ’01; Park et al ’01; Whitworth et al ’09).
  - **NLS-CAG-eGFP**: (NSRRC Unpublished)
  - **CAG-Tomato**: (NSRRC Unpublished)

Minnesota Miniature pigs expressing Tomato (left) or NLS-eGFP (right) with UV excitation (left) or normal (right) exposure.

Born 1/17/2012
Swine Gene Modifications:

- Cell Tracking/Tools
  - \textit{eGFP}: (Cabot et al ’01; Park et al ’01; Whitworth et al ’09).
  - \textit{NLS-CAG-eGFP}: (NSRRC Unpublished)
  - \textit{CAG-Tomato}: (NSRRC Unpublished)
  - \textit{eGFP-Proteasome}: (PSMA1) : (NSRRC) P. Sutovsky, MU (O’Gorman et al ‘10)

- Human/Pig Hybrid Organs -Suicide/Pro-drug System
  - \textit{Alpha fetoprotein promoter- cytosine deaminase}: (Beschorner et al ’03a) Ximerex, Inc.
  - \textit{Albumin promoter – thymidine kinase}: (Beschorner et al ’03b) Ximerex, Inc.

Born July 29, ’02
Distributed by NSRRC
Other Modifications Currently Underway at MU

- **FGF8 Conditional KO**: (Heart Development): Anne Moon, U. Utah
- **Mammary Tumor**: (NSRRC) Geoffrey Clark, U. Louisville
- **GUCA1A: Y99C and L151F, retinal degeneration**: (NSRRC) Maureen McCall, U. Louisville
- **ZP3 – Cre – LoxP/eGFP Tool Pig**: (NSRRC) K. Wells, MU
- **IFNRA1 KO, IFNγ KO, and RAG1 KO**: MU/Plum Island
- **CD163 KO**: (PRRSV resistance) MU/Plum Island
- **MSTN KO**: (Myostatin): Pfizer, K. Wells; A. Dilger, U. Illinois
- **RAG2 & IL2RG TALEN KO**: (Xenotransplantation) Kon-Kuk University, Korea
Conclusions

• Swine can be useful for Comparative Medicine as in many cases they model the human condition.

• Many types of genetic modification can be completed in pigs that have applications in:
  – Medicine
  – Production Agriculture

• We are limited only by our imagination !!
Current Projects/Grants–

- NIH U42 RR18877/OD011140 - National Swine Resource and Research Center (RSP, PI)
- NIH U42 RR18877/OD011140 – National Swine Resource and Research Center Administrative Supplement to clone pigs for xenotransplantation (RSP, PI)
- NIH R21 NS078299 - Large Animal Model of Spinal Muscular Atrophy (Monique Lorson, PI)
- NIH P01 HL51670 Iowa PPG – Gene Therapy for Cystic Fibrosis Lung Disease (Subcontract from Michael Welsh, University of Iowa)
- NIH U01 HL102288 Iowa – Iowa Phase II Clinical Trials of Novel Therapies for Lung Diseases. (Subcontract from L. Durairaj, University of Iowa)
- NIH R01 RR13438 - Differential Methylation Hybridization of Nuclear Transfer and Normal Embryos (RSP, PI)
- Korean Government via Kon-Kuk University – GGTA1 and CMAH KO’s. (RSP, PI)
- USDA ARS 58-1940-5-519 - Program for the Prevention of Animal Infectious Diseases and Advanced Technologies for Vaccines and Diagnostics (M. McIntosh, PI)
- Christopher Columbus Foundation – CD163 KO. (RSP, PI)
- NIH R01 HD069979 – Induced pluripotent stem cells from swine… (R.M. Roberts, PI)
Current Lab Members

- Dr. Clifton Murphy
- Dr. Kiho Lee
- Dr. Jiude Mao
- Dr. Kim Tessanne
- Dr. Eric Walters
- Dr. Kristin Whitworth
- Dr. Jeff Whyte
- Bethany Bauer
- Alana Brown
- Keith Giroux
- Melissa Samuel
- Lee Spate
- Armidea Stump
- Jennifer Teson
- Mingtao Zhao
- Carli Carter
- Alyssa Davis
- Lindsay Kelso
- Rebecca Mattucks
- Elaine Martin
- Jacob Miller
- Megan Pemberton
- Chris Perry
- Maren Ritterling
- F. Santibanez
- Jon Sarno
- Alyssa Thomas
- Destany Wilson

Others on Campus

- Dr. Jon Green
- Dr. Monique Lorson
- Dr. Peter Sutovsky
- Dr. Kevin Wells
- Ben Beaton
- Shasta Cernea
- Susan Cushing
- Tina Egen
- Chad O’Gorman