Transgenic pigs as models for translational biomedical research

Eckhard Wolf
Lehrstuhl für Molekulare Tierzucht und Biotechnologie und Laboratorium für funktionale Genomanalyse (LAFUGA) Genzentrum der Ludwig-Maximilians-Universität München
The translational gap

- Structural biology
- Modeling
- Biobanking
- -OMICS
  - Genomics
  - Transcriptomics
  - Proteomics
  - Metabolomics
- Theranostics
- Imaging technologies
- Preclinical models
  - Fly, Fish, Mouse
  - Pig, Sheep
  - Dog, NHP

G. Wess, 2008
The pig as model for human health research

- Monogastric omnivore with many anatomical and physiological similarities to human
- Diagnostic, surgical and many other procedures can be directly transferred to human clinical practice
- Favorable reproductive characteristics for a model organism
- Selection produced phenotypes extremely relevant to current human health research priorities, such as obesity, diabetes, cardiovascular disease
- Genetic modification is well established ➔ animal models tailored for specific questions (3R-compatible)
Genetic and phenotypic variance among different pig breeds

Meishan  Duroc
Mangalitsa  Pietrain
Favorable reproductive biology of pigs

- Pregnancy length: 115 days
- 10 to 12 piglets per litter
- > 2 litters per year
- Sexual maturity at 6 months
- Protocols established for
  - artificial insemination
  - embryo transfer
  - cryopreservation
  - etc.
Pig whole genome sequences will be available in the near future

Wernersson et al., BMC Genomics 2005, 6:70
Routes for the genetic modification of pigs

Efficient transgenesis by SCNT

- Selectable expression vector
- Transfection
- Selection
- Nuclear transfer I
- Embryo transfer
- Expressing transgenic pigs
- Embryo transfer
- Nuclear transfer II
- Establishment of cell cultures
- Fetuses or offspring
- Screening (integration, expression)

Diabetes: medical timebomb?

Dramatic increase in the prevalence of diabetes mellitus
Metabolic syndrome

Diabetes mellitus, impaired glucose tolerance, impaired fasting glucose or insulin resistance, AND two of the following:

- blood pressure $\uparrow$
- dyslipidaemia
- central obesity
- microalbuminuria
Pathogenetic principles of type 2 diabetes

> Insulin resistance
> 
> β-cell failure

The incretin effect

mediated by

Glucose-dependent insulinotropic polypeptide (GIP)
and
Glucagon-like peptide 1 (GLP-1)
GIP actions in multiple tissues

- Bone
  - Bone formation
  - Bone resorption
- Brain
  - Progenitor cell proliferation
- Adipose tissue
  - Lipogenesis
- Pancreas
  - Insulin secretion
  - Insulin biosynthesis
  - β-cell proliferation
  - β-cell apoptosis
Insulinotropic effects of GLP-1 and GIP

Müssig et al., Diabetologia, 2010
Incretin effects in type 2 diabetes mellitus

Incretin secretion

GLP-1: unchanged
GIP: mostly unchanged

Impaired GIPR signaling in type 2 diabetes

Insulinotropic effect

GLP-1: unchanged
GIP: highly reduced

Are there direct effects of impaired GIPR function on islet structure and survival?

Nauck et al., Diabetes, 2004
Genetic variation in *GIPR* influences the glucose and insulin responses to an oral glucose challenge
Glucose Intolerance and Reduced Proliferation of Pancreatic β-Cells in Transgenic Pigs With Impaired Glucose-Dependent Insulinotrophic Polypeptide Function

Simone Renner,1 Christiane Fehlings,1 Nadja Herbach,2 Andreas Hofmann,3 Dagmar C. von Waldthausen,1 Barbara Kessler,1 Karin Ulrichs,4 Irina Chodnevskaja,4 Vasilyi Moskalenko,4 Werner Amselgruber,5 Burkhard Göke,6 Alexander Pfeifer,3,7 Rüdiger Wanke,2 and Eckhard Wolf1

A

B

C

D

10 kb
8 kb
6 kb
5 kb
4 kb
3 kb
2.5 kb

F0
F1 (Sire 50)
F1 (Sire 51)
Glucose metabolism in 11-week-old pigs

**OGTT**

**AUC:**
- wt (n=5): 24028 ± 1519
- tg (n=5): 19248 ± 1402; p < 0.05

**IVGTT**

**AUC:**
- wt (n=6): 13562 ± 359
- tg (n=6): 14315 ± 1402; p = 0.663
Glucose metabolism in 5-month-old pigs

**OGTT**

![Graph a](image1)

- Time (minutes)
- Glucose (mg/dl)
- AUC: 28984 ± 1414 vs. 23856 ± 847; p < 0.05

**IVGTT**

![Graph d](image2)

- Time (minutes)
- Glucose (mg/dl)
- AUC: 14252 ± 1262 vs. 12733 ± 868; p=0.165

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**b**

![Graph b](image3)

- Time (minutes)
- Insulin (µU/ml)
- AUC: 6984 ± 716 vs. 14019 ± 1935; p < 0.01

**c**

![Graph c](image4)

- Time (minutes)
- Glucose (mg/dl)
- AUC: 1634 ± 191 vs. 1982 ± 243; p=0.106
Glucose metabolism in 11-month-old pigs

AUC: $24802 \pm 633$ vs. $22536 \pm 509$; $p < 0.05$

AUC: $2782 \pm 275$ vs. $5806 \pm 1272$; $p < 0.05$
Impaired GIP function results in a lack of physiological expansion of β-cell mass.
Reduced proliferation rate of β-cells

**a**
Ki67
Insulin

**b**

<table>
<thead>
<tr>
<th></th>
<th>11 weeks</th>
<th>5 months</th>
<th>1-1.4 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ki67+ nuclei/10^5 nuclear profiles</td>
<td>6000</td>
<td>5000</td>
<td>4000</td>
</tr>
</tbody>
</table>

*p* = 0.549

**c**
Cleaved caspase-3
Insulin

**d**

<table>
<thead>
<tr>
<th></th>
<th>11 weeks</th>
<th>5 months</th>
<th>1-1.4 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casp3+ nuclei/10^5 nuclear profiles</td>
<td>20</td>
<td>15</td>
<td>10</td>
</tr>
</tbody>
</table>

*p* = 0.313

* * *

*p* = 0.336

*p* = 0.075
Conclusions (i)

• GIPR\textsuperscript{dn} transgenic pigs demonstrate an essential role of GIPR signaling for the physiological age-related expansion of $\beta$-cell mass

• GIPR\textsuperscript{dn} transgenic pigs represent key characteristics of type 2 diabetes mellitus
  - impaired insulinotropic action of GIP
  - reduced glucose tolerance
  - impaired insulin secretion
  - progressive reduction of $\beta$-cell mass

Renner et al., Diabetes 59:1228–1238, 2010
In the design of therapeutics to treat type 2 diabetes, researchers have exploited the observation that oral ingestion of nutrients leads to the secretion of glucose homeostasis-regulating incretin hormones (for example, glucagon-like-peptide–1) from the gut. Here, we discuss two recent papers that suggest that the “other” incretin hormone, gastric inhibitory polypeptide (GIP), also is important in the regulation of glucose homeostasis. These findings warrant further studies to unravel the mechanism of action of GIP in β-cells of the endocrine pancreas and to evaluate the possibility of designing novel therapeutics that target both incretin hormones.
Conclusions (ii)

- GIPR$^{dn}$ transgenic pigs represent a highly interesting new animal model for a plethora of applications in diabetes research, including
  - Evaluation of incretin modulatory therapies (GLP-1R agonists, DPP-4 inhibitors etc.)
  - Development of in vivo imaging techniques to assess the total islet cell mass and functionality
  - Metabolomic screening for biomarkers associated with pre-diabetes and progression to clinical disease
Incretin-based therapies

- **Incretin mimetics**
  - Byetta®/Exenatide; *Lilly/Amylin*
  - Victoza®/Liraglutide; *Novo Nordisk*

- **DPP-4 inhibitors**
  - Januvia®/Sitagliptin; *Merck*
  - Galvus®/Vildagliptin; *Novartis*

Validation of GIPR$_{dn}$ transgenic pigs as a model

- Effect on insulin secretion and glucose control
- Effect on pancreatic islet mass
Need for non-invasive imaging of islet mass

• Dynamics of β-cell mass and function in diabetic patients
  ➔ Monitor loss of functional β-cell mass
  ➔ Vascularity and innervation of islets
  ➔ Autoimmune attack of islets

• Efficacy of islet (xeno)transplantation to type 1 diabetic patients
  ➔ Effects of immune rejection, glucose toxicity, and islet purity on graft fate
  ➔ Effects of transplantation site
  ➔ Effects of matrices and scaffolds
What makes us different?

Helmholtz Zentrum München
German Research Center for Environmental Health

Karsten Suhre et al.
Genotyping arrays

Metabolomics kits

HelmholtzZentrum münchen
German Research Center for Environmental Health

Karsten Suhre et al.
High throughput targeted metabolomics

amino acids

hexose

diacyl-glycero-phosphatidylcholines

acylcarnitines

acyl-alkyl-glycero-phosphatidylcholines

sphingomyelins

lyso-phosphatidylcholines

163 analytes/sample

Karsten Suhre et al.
A genome-wide perspective of genetic variation in human metabolism

Thomas Illig1,13, Christian Gieger1,13, Guangju Zhai2, Werner Römisch-Margl3, Rui Wang-Sattler1, Cornelia Prehn4, Elisabeth Altmäier3,5, Gabi Kastenmüller3, Bernet S Kato2, Hans-Werner Mewes3,6, Thomas Meitinger7,8, Martin Hrabé de Angelis4,9, Florian Kronenberg10, Nicole Soranzo2,11, H-Erich Wichmann1,12, Tim D Spector2, Jerzy Adamski4,9 & Karsten Suhre3,5

- 1,029 male and female individuals of Southern German origin from the KORA F4 population
- Affymetrix 6.0 GeneChip array (for testing 517,480 SNPs; MAF > 10%)
- 163 metabolite concentrations and all possible ratios (163 × 162 = 26,406 traits)
Biological validation in large animal models of metabolic disease

- Known time course of disease progression
- Defined nutritional management
- Knowledge of co-morbidities
- Repeated sampling of sufficient volumes of biological substrates
- Metabolic challenge tests (tolerance tests, stimulation tests, clamp studies)
- …

⇒ Ideal for the discovery of biomarkers for disease progression
Metabolic screening of GIPR<sup>dn</sup> transgenic pigs and littermate controls (samples from IVGTT)

8 animals (4 tg, 4 co) x 3 age classes x 11 repeats x 146 parameters

<table>
<thead>
<tr>
<th>Metabolite class (n measured)</th>
<th>n parameters significantly affected by</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group</td>
</tr>
<tr>
<td>Acyl-carnithins (38)</td>
<td>11</td>
</tr>
<tr>
<td>Amino acids (14)</td>
<td>1</td>
</tr>
<tr>
<td>Phosphatidylcholins (82)</td>
<td>2</td>
</tr>
<tr>
<td>Sphingomyelins (12)</td>
<td>0</td>
</tr>
<tr>
<td>Total (146)</td>
<td>14</td>
</tr>
</tbody>
</table>
Metabolic screening of GIPR\textsuperscript{dn} transgenic pigs and littermate controls (samples from IVGTT)

8 animals (4 tg, 4 co) $\times$ 3 age classes $\times$ 11 repeats $\times$ 146 parameters

Example: Acyl-carnitines in 5-month-old animals
Search for target genes and mechanisms

Human patients

G A T C A A T G T
C T A G T T A C A

GWA studies

G A T C A T T G T
C T A G T A A C A

Pathophysiology

G A T C T A T G T
C T A G A T A C A

Model organisms

Forward genetics

Reverse genetics

Cellular systems

Development and evaluation of POC animal models

Ko 1

Ko 2

Tg 1

Tg 2

Mut 1

Mut 2

Development of advanced preclinical animal models for evaluation of safety and efficacy

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