CRISPR/Cas9-Directed Inactivation of Porcine Interleukin-1β as a Model to Study Endometrial Receptivity and Conception Attachment

Jeffrey J. Whyte, Madison E. Hennessy, Rodney D. Geisert, and Randall S. Prather

Abstract
An understanding of successful pregnancy establishment in pigs is important for translational research and commercial pig production. Failure of conceptus-maternal communication is a major contributor to embryonic loss, yet its molecular control is unclear. Engineered nucleases such as the bacterial clustered regularly interspaced short palindromic repeat (CRISPR/Cas9) or CRISPRi system enable precise genome modification. We hypothesize that this system can be used to produce genetically-modified pig embryo donor cells to be used for somatic cell nuclear transfer (SCNT) and IL1BL were targeted in fetal fibroblast cells. These cells will be used to produce SCNT embryos to reveal the impact of IL1BL disruption on critical molecular pathways and their role in successful pregnancy establishment.

Introduction
Embryonic losses in swine and most mammals ranges from 30% to 40%.

Porcine interleukin-1β forms
- IL1BL Conceptus: In conceptus at peri-implantation
- IL1BL Systemic: In macrophages and endometrium

Porcine IL1B Paralogs
- IL1BL Conceptus: Targets at 1 exon
- IL1BL Systemic: Targets at 2 exons

Hypothesis
Porcine IL1B knockout embryos models produced with engineered nucleases will answer key questions about the complex conceptus-maternal molecular dialogue.

Specific Objectives
1. Develop CRISPR/Cas9 nucleases to inactivate IL1BL and IL1B in pig fibroblasts.
2. Create SCNT embryos from IL1BL−/−, IL1B−/−, and wild-type donor fibroblasts.
3. Identify key molecular effects of IL1BL/IL1B knockout on early pig development.

Proposed role of IL1BL in maternal recognition signaling pathway between the developing conceptus and endometrium.

Luminal Epithelium
- IL1BL
- Conceptus
- PGs modulation
- Nutrient growth factors

Endometrial inflammatory pathways
- NF-κB Activation
- E2-induced genes
- Endometrial remodeling, cell adher.

Conceptus
- IL1R1
- IL1β
- P4-induced uterine recept.
- Inhibition of NF-κB
- Pregnancy recognition
- E2-induced genes
- Transport

P4-induced孕酮

E2, estrogen; IL1BL, conceptus interleukin-1β; IL1R1, interleukin-1 receptor; PGs, prostaglandins; P4, progesterone; NF-κB, nuclear factor kappaB (modified from Bauersachs and Wolf, 2012, Geisert et al. 2014).

Validate Gene Sequences in Porcine Donor Cells
Verify that the nuclear transfer donor cell IL1BL and IL1B gene sequences match the published genome sequence in the regions to be targeted for disruption.

Identify Unique sgRNA for IL1BL and IL1B Targets
Restrict search to first two coding sequences.

Genomic DNA (e.g. IL1B)
Genomic DNA (e.g. IL1B)

CRISPR/Cas9 Gene Disruption
CRISPR/Cas9 with an sgRNA complementary to a 20-bp target sequence (protospacer) and the 5′-NGG-3′ (protospacer adjacent motif; PAM).

Double strand breaks can lead to base insertions or deletions through errors in non-homologous end-joining (NHEJ).

Procedures

Results

IL1BL Unique sgRNA
IL1BL CRISPR sgRNA in vitro verification
IL1BL CRISPR sgRNA cleavage of porcine IL1BL target PCR product
In vitro targeting of porcine IL1BL with IL1BL CRISPR sgRNAs

IL1B Unique sgRNA
IL1B CRISPR sgRNA in vitro verification
IL1B CRISPR sgRNA cleavage of porcine IL1B target PCR product
In vitro targeting of porcine IL1B with IL1B CRISPR sgRNAs

Conclusions
We have demonstrated in vitro targeting of porcine IL1BL and IL1B with the CRISPR/Cas9 system. CRISPRi sgRNAs for IL1BL did not cleave IL1B, and vice versa, verifying that the sgRNAs for each gene are highly specific to their respective targets. Cultured pig donor cells electroporated with sgRNA pairs bracketing the start codon of each gene form are being selected for clonal populations of targeted cells. These cells will be used to produce SCNT embryos to reveal the impact of IL1BL/IL1B disruption on critical molecular pathways and their role in successful pregnancy establishment in pigs.

Acknowledgments
This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2013-67015-21023 from the USDA National Institute of Food and Agriculture. The authors are grateful to Dr. Chris Tuggle and Dr. Martine Schroyen (Iowa State University) for IL1BL sequence data, and to Dr. Kristin Whithworth, Lee Spalte, Melissa Samuel, Ben Beaton and Dr. Clifton Murphy for their assistance in this project.