Study on the feasibility of recombinant protegrin expression and secretion in tracheal epithelial cells

R.J. DeVries, P.W. Dyce, H. Miller, J.Peacock and J. Li
Department of Animal and Poultry Science, University of Guelph
519-824-4120 ext. 52713, jli@uoguelph.ca

Background

Extensive use of antibiotics for treatment and prophylactic treatment against infection in the livestock industry is suggested to link to increases in antimicrobial resistance. Searching for alternative means of preventing and treating emerging and re-emerging diseases has become a new challenge to overcome antibiotic resistance and thus to retain consumer confidence in a safe food supply. Antimicrobial peptides (AMPs) are "natural antibiotic" involved in innate immunity. Antimicrobial peptides generally possess a broad spectrum of activity including various bacteria, fungi, protozoa, and in some cases, enveloped viruses. In the pig, the most potent form of antimicrobial peptide is protegrin (PG) and is produced by the neutrophils. The long term goal of the research is to produce protegrin in the respiratory tract of transgenic pigs and thus potentially reduce the need for antibiotics in the pork industry. The current pilot project is to determine the feasibility of expressing protegrin 1 in the porcine tracheal epithelial cells in vitro.

Specific Objectives

To isolate protegrin 1 cDNA, construct and optimize a vector for protegrin-1 expression
To develop an isolation and culture method for porcine tracheal epithelial cells (TEC)
To test the expression and antimicrobial activity of protegrin-1 in TEC

Results

Recombinant Protegrin1 is transcribed within porcine tracheal epithelial cells (Figure 1). Removal of the synthetic intron from the expression vector resulted in an over 15 fold increase in transcription as detected by real-time PCR (Figure 2).
Despite this increase in expression and the optimization of transfection methods, antimicrobial activity could not be consistently detected in TEC via radial diffusion assays of the cell culture medium.

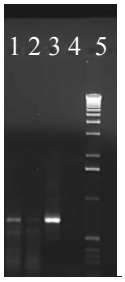

Figure 1: A representitive photograph showing the detection of protegrin transcripts in porcine tracheal epithelial cells via RT-PCR.
Lane 1: transfected cells 24 hours post-transfection
Lane 2: transfected cells 48 hours post-transfection
Lane 3: femur bone marrow cells as a positive control
Lane 4: RT-PCR negative control
Lane 5: 1 Kb DNA ladder

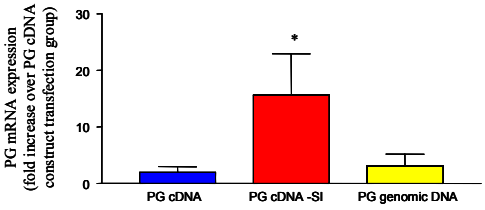

Figure 2: PG mRNA from various transfection groups were analyzed via real-time PCR. Data are expressed as fold increased comparing to the expression in cells using PG cDNA in the expression construct (Blue bar). Red bar: construct with PG cDNA with the deletion of the synthetic intron in the construct. Yellow bar: construct with PG genomic DNA..

Take Home Messages

Protegrin-1 is transcribed within porcine TEC. The antimicrobial activity of the expressed protegrin-1 in TEC is variable, as repetition of the experiments did not demonstrate activity consistently. The feasibility of expressing other antimicrobial peptides in tracheal epithelial cells is to be investigated.

We appreciate the financial support of Ontario Pork, Agriculture and Agri-Food Canada, NSERC, Food System Biotechnology Centre and OMAF.