

DND1 Expression and Function in the Porcine Ovary, Oocyte and Embryo

A.S. Leaflet R2740

Cai-Xia Yang, postdoctoral research associate;
Elane C. Wright, graduate research assistant;
Jason W. Ross, assistant professor,
Department of Animal Science

Summary and Implications

DND1 (dead end homolog 1), belonging to the RNA binding protein family, can impact miRNA:mRNA functional pathway and in turn may contribute to maintaining normal oocyte growth and quality as well as embryo development following fertilization. To characterize DND1 in pig maturing oocytes and cumulus cells, and early embryos, we examined DND1 mRNA and protein expression using quantitative RT-PCR, Western blot and immunostaining. We found: (1) DND1 protein is expressed during pig follicle development; (2) DND1 is dynamically expressed at both mRNA and protein level in the maturing oocyte and early in vitro fertilized embryos; (3) DND1 mRNA is expressed in cumulus cells surrounding the maturing oocyte; and (4) DND1 protein is localized in the cytoplasm of pig maturing oocytes and early embryos. Our work provides useful data for functional study of DND1 proteins in female gametogenesis and developing embryos, which will benefit animal reproduction health and provides foundational knowledge for improving swine reproductive efficiency.

Introduction

MicroRNA (miRNA) is one class of small endogenous RNA, which are expressed in tissue or cell-specific patterns, including mammalian oocytes and embryos. Endogenous miRNA can regulate both mRNA and protein repertoires through their ability to confer post transcriptional gene regulation (PTGR) through interactions with the 3' untranslated region (3'UTR) of mRNA transcripts. However, several factors influence the miRNA:mRNA interactions and they can depend largely on the expression of several critical RNA binding proteins, such as dead end homolog 1 (DND1). Our working hypothesis is that DND1 expression during porcine oocyte and embryo development influences specific PTGR events through interactions with mRNA 3'UTR, and positively influencing mRNA stability and translation during oocyte maturation and early embryo development. The purpose of this study was to determine DND1 expression pattern during pig follicle development, oocyte maturation, and early embryo development.

Materials and Methods

Sow ovaries were obtained from an abattoir and antral follicles were aspirated. Oocytes at germinal vesicle stage were collected and matured in vitro for 42-44 hours. The matured oocytes with the first polar body (MII stage) were in vitro fertilized using fresh boar sperm. Samples were collected at GV stage, MII arrest, and at the 4-8 cell stage of embryonic development for immunocytochemistry, quantitative RT-PCR and Western blotting. Sow ovaries were fixed in paraformaldehyde and embedded in paraffin and serially sectioned. Immunostaining images were captured using a confocal microscope system. The quantitative analyses of staining and Western blotting were performed using Image J software.

Results and Discussion

DND1 protein is highly expressed in secondary follicles in contrast to primary and tertiary follicles. DND1 mRNA is less abundant in the 4-cell stage embryo as compared to GV and MII oocytes. DND1 protein is highly expressed in MII oocytes as compared to GV oocytes and 4-cell embryos. DND1 mRNA is expressed in cumulus cells isolated from both GV stage and MII arrested oocytes. DND1 protein is localized in the cytoplasm of pig maturing oocytes and early embryos, but highly enriched in the nucleus of cumulus cells (Figure 1). These preliminary data establish the presence of DND1 expression during pig oocyte maturation and early embryo development. Further investigations of DND1 function in addition to identification of specific mRNA capable of interacting with DND1 that are expressed in the pig oocyte during maturation and during early embryo development is ongoing.

Acknowledgements

This project was funded in part by the National Research Initiative Competitive Grant no. 2008-35205-05309 and 2008-35205-18712 from the USDA National Institute of Food and Agriculture.

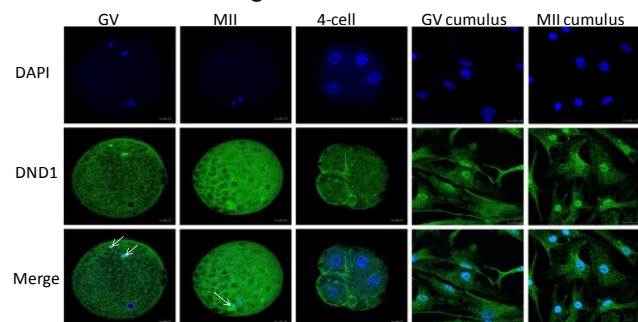


Figure 1. DND1 protein localization in maturing pig oocyte, early embryo and cumulus cells.