ACENTRIOLAR MICROTUBULE ORGANIZATION CENTERS AND RAN-MEDIATED MICROTUBULE FORMATION PATHWAYS ARE BOTH REQUIRED IN PORCINE OOCYTES

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Introduction

Mammalian oocytes lack centrioles, but can generate bipolar spindles using several different mechanisms. Full knowledge of the different mechanisms of spindle assembly in various mammalian oocytes is lacking. In this study, we show that both MTOC-mediated and Ran-mediated microtubule formation are required for the functional meiotic metaphase I spindle generation in porcine oocytes. The results demonstrate that the stepwise involvement of Ran- and MTOC-mediated microtubule assembly is crucial for the formation of meiotic spindles in porcine oocytes, indicating the diversity of spindle formation mechanisms among mammalian oocytes.

Materials and Methods

Porcine oocytes were obtained from 30~40 ovaries using 18-gauge micro needles and were transferred into *in vitro* maturation medium. Localization of MTOC and spindle were tested by immunofluorescent staining of Centrosomal protein of kDa 192 (Cep192) and α-tubulin. Function of MTOC was tested by using microinjection of Cep192 dsRNA and treatment of Polo-like kinase 1 inhibitor. Relative gene expression related with Cep192 was quantified by RT-PCR. The Cep192 and spindle protein levels were quantified using ImageJ software. The average fluorescence intensity per unit area within a region of interest was determined. Function of Ran-mediated microtubule assembly was tested by the expression of dominant negative or constitutively active Ran mutants using microinjection of cRNA.

Results and Discussion

Cep192 is localized at the spindle at Metaphase I and Metaphase II stage microtubules, but is absent at the initial microtubule polymerization at GVBD stages. Injection of dsRNA specific for porcine Cep192 depleted the Cep192 mRNA levels by as much as 20% of the levels in the negative control GFP dsRNA–injected oocytes. Treatment of the PLK1-specific inhibitor, BI-2536, decreased Cep192 and alpha-tubulin levels, similar with phenotype Cep192 knockdown. Microtubule and Cep192 fluorescence levels were severely diminished following the injection of the dominant negative or constitutively active Ran mutants. Collectively, the data demonstrate significant mechanistic differences in the spindle formation mechanism between mammalian oocytes, including human, mouse, and porcine oocytes.