

WHAMM PLAY ESSENTIAL ROLES IN SPINDLE FORMATION AND CAGE ACTIN MESH OF MATURING MOUSE OOCYTES

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Introduction

Dynamic actin filament polymerization and depolymerization is essential for various cellular processes including cell migration, rotation, cytokinesis and mammalian oocyte maturation. Various actin-binding proteins (ABPs) regulate actin cytoskeletons reorganization. WHAMM (WAS Protein Homolog Associated with Actin, Golgi Membranes and Microtubules) plays a role in actin nucleation, Golgi membrane association and microtubule binding. The WHAMM protein is a nucleation-promoting factor that activates the Actin-related protein 2/3 complex (Arp2/3) and initiates growth of new actin filaments by binding to existing actin filaments. WHAMM also functions in regulation of transport from the endoplasmic reticulum to the Golgi complex and in maintenance of the Golgi complex near the centrosome. However, the roles of WHAMM in mammalian oocyte maturation are poorly understood.

Materials and Methods

Germinal vesicle (GV)-intact oocytes were collected from the ovaries of 6–8-week-old imprinting control region (ICR) mice and cultured in M16 medium (Sigma) under paraffin oil at 37°C in 5% CO₂. Oocytes were collected in M2 medium for immunostaining and microinjection after culturing for various amounts of time. Function of WHAMM was tested by using microinjection of WHAMM dsRNA. Relative gene expression related with WHAMM was quantified by RT-PCR. The WHAMM and F-actin levels were quantified using ImageJ software. The average fluorescence intensity per unit area within a region of interest was determined.

Results and Discussion

Presence of WHAMM in mRNA and protein level have been confirmed during all stage of mouse oocyte maturation. It mainly localized in spindle pole and appears enriched in near the chromosome during maturation. By knock down, we confirmed that WHAMM is regulator for formation of spindle. And also WHAMM affect the localization of MTOC during the spindle formation. Taken together, these findings show that WHAMM is one of essential component of the actin cytoskeleton machinery that crucial roles in oocyte maturation, presumably by controlling formation of normal length spindle by activating the cage actin mesh by Arp2/3 complex.