Proc 4th Ann Conf Kor Soc Dev Biol, July 3, 1998, pp 29.

P-5 Ontogenesis of Mouse Sperm Centrioles during Spermatogenesis: Immunocytochemical and Ultrastructural Studies

Kwang Hee Lee[1,2], Hong Jun Lee[1], Jung Won Park[2], Ki Dong Kim[1] and Sang Ho Lee[1]
Graduate School of Biotechnology, Korea University, Seoul[1]
Veterinary Research Institute, Anyang[2]

INTRODUCTION

Unlike other mammals so far studied, mouse sperm centrioles do not appear to be functional from the first cleavage onwards. Therefore, it is assumed that maternal centrosomes seem to organize the first mitotic spindle (Sathananthan et al., 1997). This uncertain debate comes partly from the lack of evidences for the presence of mouse sperm centrioles and consequently no follow-up study in fertilized mouse eggs. In human, centrioles have been demonstrated in mature and penetrated spermatozoa into the eggs, suggesting paternal inheritance of centrioles in fetal and adults somatic cells (Sathannthan et al., 1996).

The first question is then "are there two centrioles in germ cells during spermatogenesis and in mature spermatozoa in the mouse?" To understand unresolved peculiarity in the mouse, it is necessary to demonstrate the presence of mouse sperm centrioles. We initiated an investigation to establish centriolar ontogenesis in developing mouse spermatozoa.

MATERIALS & METHODS

Mouse somatic and germ cells were isolated by standard techniques for immunocytochemistry. Centrioles were visualized antitubulin antibodies after extensive extraction of cells either on glass or in suspension. For ultrastructural study, appropriate tissues were processed for transmission electron microscopy. Mouse testis at 6, 8, 10, 12 and 18 days after birth and from adults were used to obtain different cell types during spermatogenesis (Bellve, 1993).

RESULTS & DISCUSSION

Although markers for the centrosomes which consists of centrioles and pericentriolar materials (PCM) has generally been used to localize spindle pole, it is not known whether the centrosomes visualized in various studies contain one, two or more centrioles. The centriole markers we used three antibodies recognize β-tubulin(β), acetylated(Ace) tubulin and tyrosine tubulin(Tub) respectively. Three antibodies all recognized centrioles very clearly in properly extracted mouse embryonic fibroblast(MEF) depolymerized at 0℃. Ace antibody gave more discrete staining, distinguishing two separate centrioles at the level of the light microscopy. It also demonstrated duplicated centrioles. Therefore this antibody was subsequently used for germ cells.

Spermatogonia and spermatocytes showed a similar result shown as in MEF. In addition to the centriole staining, spermatocytes also showed accumulated acetylated tubulin in cytoplasm probably for the preparation of axoneme structure. This was more clearly seen in developing spermatids and mature spermatozoa, when the axoneme become gradually elongated. Two centrioles at the base of spermatid sperm nucleus were clearly seen. Ultrastructural stuy confirmed the biogenesis of mouse sperm centrioles.

The results from the immnunocytochemical and ultrastuctural studies indicate that mouse sperm centrioles persist in developing spermatogenic cells and mature spermatozoa, and that a similar organization of sperm centriole may occur during fertilization.

REFERENCES

Bellve AR (1996) *Methods in enzymology* 225: 84-113.

Sathananthan (1997) *Histol Histopathol.* 12(3): 827-856.

Sathananthan (1996) *Human reprod.* 11(2): 345-356.