Introduction

Although both the spindle and nuclear mitotic apparatus (NuMA) are thought to play important roles in the cell cycle and early reprogramming after somatic nuclear transfer, the spindle and NuMA morphogenesis during the early stage after NT is not well understood.

In this study we systematically characterized the spindle morphogenesis, the correlation between the donor nuclear membrane and spindle formation, and chromosome configuration in the early stage of the first mitosis in mouse NT.

Conclusion

The spindle morphogenesis was independent of the presence or absence of a metaphase II spindle and it was initiated by a variety of spindle defects, including a high proportion of the monopolar spindle phenotype.

The phenotypes of spindle morphogenesis were correlated with intact or disruptive donor membrane.

Our study also uncovered a novel model for incomplete spindle remodeling by NT in the mouse, which occurs when a monopolar spindle is converted to a bipolar spindle or a monastral bipolar spindle lacks a pole, is therefore an incomplete spindle remodeling.

Materials and Methods

Oocytes collection and nuclear transfer:

Oocytes were collected from mature female BALB/c mice approximately 56 hr after HCG injection. The donor nucleus was introduced into a cumulus cell in the presence of 5% CO2:95% air. The cumulus cell was isolated using an aspiration pipette and the cumulus cell and nucleus were transferred into the injection pipette. The nucleus was injected into the oocyte using a small micropipette. Fixation and differential staining:

The samples were fixed in 3.5% paraformaldehyde and then kept overnight at 4 degree C in PBS-BSA containing 1% Triton X-100. For double labeling, DNA was stained with PI (red), and lamin A/C were stained with Alexa 568 (red). NuMA was detected with Alexa 488 (green). FITC, Alexa 488 (green) and Alexa 568 (red). All samples were visualized with a BioRad Radiance 2100 confocal microscope.

Embryo transfer:

Embryos were transferred into pseudo-pregnant ICR (ICR) females on day 1 of pseudopregnancy following mating with ICR vasectomized males.

Results

Initial spindle assembly (α-tubulin) in mouse somatic cell nuclear transfer

Fig. 1. The initial spindle morphologies detected in the first mitosis of mouse somatic nuclear transfer. Oocytes were fixed within 30 minutes after NT and stained by anti-α-tubulin-conjugated FITC (green). DNA was stained with propidium iodide (red). A-A′ Bipolar spindle phenotype. B-B′ Monopolar spindle phenotype. C-C′ Multipolar spindle phenotype.

The morphological change of NuMA before and after activation in the first mitosis of mouse NT oocytes/embryos

Fig. 2. The initial centrosomal morphologies detected in the first mitosis of mouse somatic nuclear transfer. Oocytes were fixed within 30 minutes after NT and stained by anti-NuMA and Alexa 488 (green). DNA was stained with propidium iodide (Red). A-A′ Bipolar centrosomal phenotype. B-B′ Monopolar centrosomal phenotype. C-C′ Multipolar centrosomal phenotype.

The morphology of NuMA before and after activation in the first mitosis of mouse NT oocytes/embryos

Fig. 3. The spindle morphogenesis in mouse nuclear transfer after activation of cumulus cell in to an intact or enucleated oocyte. A total of 920 oocytes (420 intact MB oocytes and 500 enucleated oocytes) were injected with cumulus cells and fixed at 10, 20, 30 min after NT. The initial spindle morphology of donor nucleus injected into intact oocytes and enucleated oocytes could be determined in 365 and 425 oocytes, respectively.

Effects of intact and enucleated oocytes on the spindle morphogenesis after nuclear transfer

Fig. 4. The first mitotic metaphase after injection of a somatic nucleus into an intact (A) or enucleated oocyte (B). C) A metaphase II oocyte with chromosomes located at the equatorial region of the spindle. D) During the first mitotic metaphase of mouse NT, chromosomes were not located in the equatorial region of the metaphase spindle, they tended to be located in a polar region of the spindle.

Disruption of nuclear membrane using a small micropipette

Fig. 5. Centrosomal morphologies at the first mitotic metaphase of mouse NT. Nuclear transferred oocytes were fixed 60 minutes after NT. NuMA was stained with Anti-NuMA and Alexa 488 (green). DNA was stained with propidium iodide (Red). A-A′ A bipolar spindle morphology at the first mitosis of mouse NT with two static NuMA foils adhered with microtubule-organizing centers (NuMA-MTOCs) was detected. B-B′ A monopolar spindle morphology at the first mitosis of mouse NT with only one NuMA-MTOC was detected. C-C′ A Bipolar spindle morphology of mouse NT. II oocytes with two NuMA-MTOCs was detected (Control).

Effects of intact or disrupted nuclear membrane on the spindle morphogenesis in mouse somatic nuclear transfer

Fig. 6. More than 600 nuclear transferred oocytes at 5-minute intervals from 10 to 40 minutes after NT and stained them with α-tubulin and NuMA to observe the transformation of monopolar to bipolar spindle in NT of mice. The conversion of a monopolar spindle to a bipolar spindle by bipolarization of a centrosome was determined.

Transformation from monopolar to bipolar spindle in mouse somatic nuclear transfer

Fig. 7. To disrupt the nuclear membrane, we drew cumulus cells in and out of the injection pipette while decreasing the diameter of the injection pipette from 8 to 4 µm (A, A′). To confirm whether the cumulus cell nuclear membrane was disrupted, we fixed nuclear transferred oocytes immediately after injection and stained them with α-tubulin and NuMA. A′) A monopolar spindle was formed. B) An intact cumulus cell nuclear membrane was disrupted, a monopolar spindle was formed (control). C) An intact cumulus cell nuclear membrane by a 2.3 µm micropipette. D) A disruption of microspere.

Disruption of nuclear membrane using a small micropipette

Fig. 8. The frequencies of initial spindle morphologies after injection of somatic nuclei into enucleated oocytes by micropipettes of different diameters. This experiment was repeated four times to obtain 160 nuclear transfer oocytes per treatment.

Table 1. Only two cloned mice (1.8%) were obtained after we transferred 116 of the 2-cell embryos from the 5.4 µm pipette to the recipient mouse.

The model of the first mitotic spindle morphogenesis in mouse nuclear transfer

Fig. 9. A) Bipolar pathway, in which donor nuclear membrane was broken down and α-tubulin was assembled at the poles of the spindle. The α-tubulin was prolonged, there was chromosome condensation, and chromosomes were ultimately located at the equatorial region of the spindle.

Full term development

Fig. 10. The development of 2-cell embryos (Live) from two-cell embryos (Dead).

Discussion of nuclear membrane using a small micropipette

SPINDLE MORPHOGENESIS AND THE MORPHOLOGY OF CHROMOSOME IN MOUSE NUCLEAR TRANSFER: AN ABNORMAL START IN CLONING OF MICE

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