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Survivin regulates Plk1 localization to kinetochore in mouse oocyte meiosis

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ABSTRACT

Survivin is a member of inhibitors of apoptosis proteins (IAPs), and also belongs to be a member of the chromosomal passenger complex (CPC) which has multiple functions including inhibition of apoptosis and regulation of cell division and SAC activity. Plk1 (polo-like kinase 1) associates with the spindle poles and also distributes to the kinetochores and is shown to involve in spindle organization, APC/C activation and cytokinesis in many models. Our recent work has shown that Survivin is a critical regulator of chromosome segregation and spindle assembly checkpoint (SAC) in meiosis. In the present study, we found that Plk1 co-localized with Survivin at metaphase I (MI) and telophase I (TI) stage after GVBD. Plk1 dispersed into the oocyte cytoplasm or accumulated near the chromosomes after the depletion of Survivin by morpholino (MO) injection. Our results showed that the localization of Plk1 to kinetochores required the involvement of Survivin.

1. Introduction

Mammalian meiosis is different from mitosis in that the former includes two cell divisions, meiosis I and meiosis II. The chromosomes duplicate once and divide twice: In meiosis I, paired homologous chromosomes are segregated, and in meiosis II sister chromatids are segregated in a process similar to mitosis. Spindle assembly checkpoint (SAC) is the mechanism which can ensure precise chromosome segregation by inhibiting the activity of the anaphase promoting complex/cyclosome (APC/C) in mitosis, and this mechanism is also proved to exist in meiosis [1]. SAC is activated both by unoccupied kinetochores or kinetochores with incorrect microtubule attachment tension.

Survivin is a member of inhibitors of apoptosis proteins (IAPs), which have multiple functions including inhibition of apoptosis and regulation of cell division and SAC activity [2]. Survivin also belongs to be a member of the chromosomal passenger complex (CPC), which also includes Aurora B, inner centromere protein (INCENP), and Borealin/Dasra B. The functions of CPC are to correct the misaligned chromosomes, properly form the central spindle, and complete cytokinesis. The role of Survivin in the CPC is to target the other members of CPC to the different locations during cell division [2]. Survivin is one molecule which was shown to regulate SAC activity in mouse oocyte meiosis. Our previous work shows that Survivin localizes at kinetochores, regulates oocyte chromosome segregation, spindle formation and SAC activity in meiosis [3].

Plk1 (polo-like kinase 1) is distinct serine/threonine protein kinase and belongs to polo-like kinase family. Plk1 associates with the spindle poles and also distributes to the kinetochores from prophase until anaphase. Plk1 is shown to involve in spindle organization, APC/C activation and cytokinesis in many models [4]. Recent work also proves that Plk1 also regulates SAC activity and associates with SAC regulators like Mad1 and Mps1 [5]. In mouse oocytes, Plk1 interacts with MAPK signal pathway to involve in spindle organization and cell cycle progression [6].

Although roles of Plk1 in mouse oocyte have been uncovered, the complete signal pathway of Plk1 in meiosis is still poorly understood. Since Survivin and Plk1 all involve in the spindle formation, chromosome alignment, SAC activity and cytokinesis, their similar roles make us to investigate the relationship between Survivin and Plk1. And our results identified that Survivin is an upstream regulator of Plk1 in meiosis.

2. Material and methods

2.1. Antibodies

Rabbit monoclonal anti-Survivin antibody was purchased from Cell Signaling Technology (Beverly, MA); mouse monoclonal anti-Plk1 antibody was purchased from Abcam (Cambridge, MA). All secondary antibodies were purchased from Zhongshan Golden Bridge Biotechnology Co., LTD (Beijing).

2.2. Oocyte collection and culture

All animal manipulations were conducted according to the guidelines of the Animal Research Committee of the Institute of

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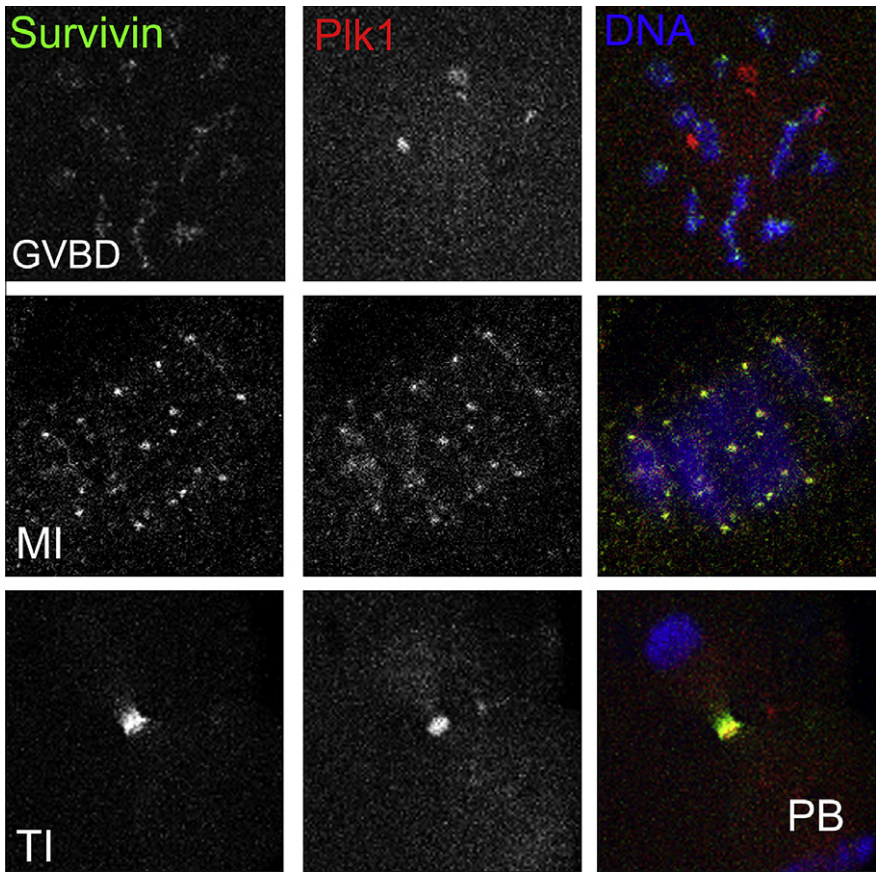

Fig. 1. Localization of Survivin and Plk1 in mouse oocyte meiosis. After GVBD, Survivin accumulated to the kinetochores, while Plk1 localized around the chromosomes; MI (metaphase I) stage, Survivin and Plk1 were shown to co-localize at the kinetochores; TI (telophase I) stage, Survivin and Plk1 co-localized at the midbody. Green, Survivin; red, Plk1; blue, chromatin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

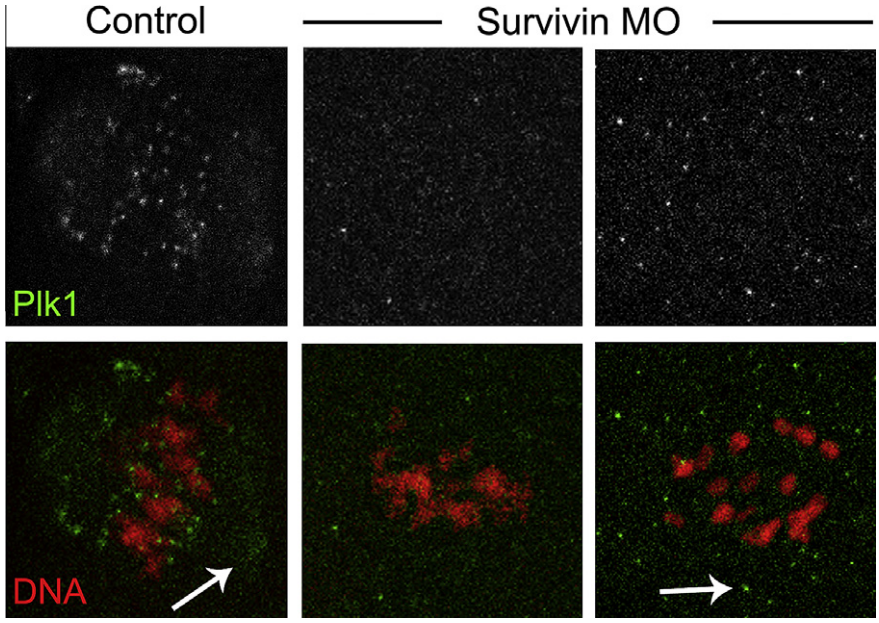

Fig. 2. Localization of Plk1 after depletion of Survivin. In the control oocyte, Plk1 localized at the kinetochores and spindle poles; while in the Survivin-deleted oocytes, Plk1 was dispersed into the cytoplasm or near the chromosomes as dots. Green, Plk1; red, chromatin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Zoology, Chinese Academy of Sciences. Germinal vesicle-intact oocytes were collected from ovaries of 6-8 week-old ICR mice. They were cultured in M2 medium (Sigma Chemical Co., St. Louis, MO) under paraffin oil at 37 °C, 5% CO_2 in air. Oocytes were collected at different times of culture for immunostaining and microinjection.

2.3. Morpholino injection

For Survivin knock down in mouse oocytes, survivin MO 5′-GTC ACC ACA ACC TCC GCC AAG ACG A-3′ (Gene Tools, LLC) was diluted by water (Sigma Chemical Co., St. Louis, MO) to give a concentration of 2 mM. Fully grown GV oocytes were microinjected with 5-10 pl MO (Gene Tools, LLC) in M2 medium containing 2.5 μM Milrinone (Sigma) for preventing oocyte GV breakdown. Oocytes were incubated in M2 medium containing 2.5 μM Milrinone for 24 h, washed 5 times in fresh M2 medium, and then cultured in fresh M2 medium to resume meiosis. The oocytes cultured for 8 h (for MI stage) were collected for subsequent experiments. The control was injected with MO standard control 5′- CCT CTT ACC TCA gTT ACA ATT TAT A-3′.

2.4. Confocal microscopy

The method was adopted as previously described [7,8]. Oocytes were fixed in 4% paraformaldehyde in PBS for 30 min at room temperature. Then they were transferred to membrane permeabilization solution (0.5% Triton X-100) for 20 min. After 1 h block in blocking buffer (1% BSA-supplemented PBS), oocytes were incubated overnight at 4 °C or 4 h at room temperature with 1:800 rabbit anti-Survivin or 1:200 anti-Plk1 antibody. After 3 washes in washing buffer (0.1% Tween-20 and 0.01% Triton X-100 in PBS), they were labeled with 1:100 secondary antibody for 1 h at room temperature. They were co-stained with Hoechst or PI for 5 min with 3 additional washes in washing buffer.

2.5. Data analysis

For each treatment, at least three replicates were performed. Statistical analyses were conducted by analysis of variance. Differences between treated groups were evaluated with the Duncan multiple comparison test. Data are expressed as mean ± SEM and $P < 0.05$ is considered significant.

3. Results

3.1. Co-localization of Survivin and Plk1 in meiosis

To explore the relationship between Survivin and Plk1 in meiosis, we first examined the co-localization of Survivin and Plk1 during mouse oocyte maturation. We found that Survivin localized at the kinetochores after GVBD (germinal vesicle breakdown), while Plk1 accumulated around the chromosomes. This localization pattern is similar with the previous work [9]. When the oocyte reached metaphase I (MI), Survivin and Plk1 were shown to co-localize at the kinetochores. At telophase I (TI), Survivin and Plk1 also co-localized at the midbody (Fig. 1).

3.2. Depletion of Survivin disrupts kinetochore localization of Plk1 in meiosis

To confirm our hypothesis, we knocked down Survivin by morpholino (MO) injection in mouse oocytes. After the expression of Survivin decreased (see our previous work [3]), the chromosomes misaligned at MI stage. We found that the localization of Plk1 was disrupted in the Survivin-deleted oocytes. In the control oocyte, Plk1 localized at the spindle pole and kinetochores. However, in the Survivin-deleted oocytes, two phenotypes were found: first, Plk1 dispersed into the cytoplasm, and no specific signal was observed; second, Plk1 failed to localize to the kinetochores, instead, it accumulated near the chromosomes as dots (Fig. 2). And the rate of abnormal Plk1 localization pattern was 82.6 ± 7.5% ($n = 34$), significantly higher than that of the control group (15.9 ± 1.4%, $n = 25$) ($p < 0.05$). We also analyzed the localization pattern composition, and the results showed that the rate of Plk1 lost from the kinetochores was 54.8 ± 8.9%, while the rate of Plk1 near the kinetochores was 27.8 ± 6.9% (Fig. 3).

4. Discussion

In the present study, we investigated the relationship of Survivin and Plk1 during mouse oocyte meiotic maturation. The results demonstrated Survivin and Plk1 co-localized at metaphase I and telophase I stage in oocytes. In particular, the disruption of the activity of Survivin by Morpholino injection approach can affect the kinetochore localization of Plk1. The study therefore provides direct evidence that Survivin is an upstream regulator for Plk1 in meiosis.

Survivin and Plk1 co-localized at MI stage. This localization pattern indicated that after GVBD, Survivin might load to kinetochores first to recruit Plk1; and after the recruitment of Plk1 to the kinetochores, Survivin associated with Plk1 and involved into spindle formation, chromosome alignment and SAC activity at MI stage, which has been shown by the previous work [3,10]. And the co-localization of Survivin and Plk1 at TI stage indicated that these two molecules might involve in cytokinesis, as the midbody is critical for polar body emission [11].

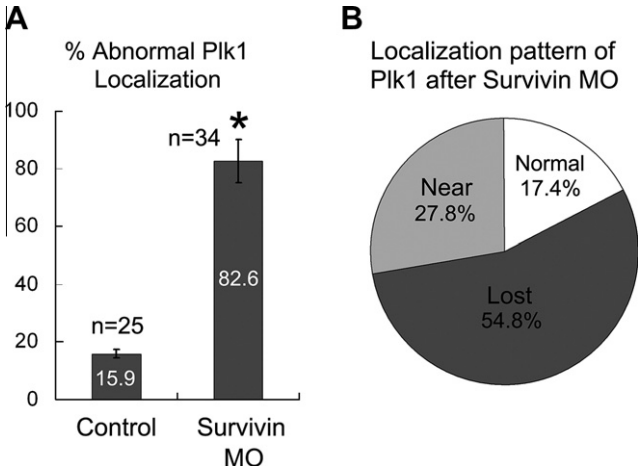

Fig. 3. (A) Rate of abnormal Plk1 localization pattern after Survivin MO injection. (B) Composition of Plk1 localization pattern after Survivin MO injection.

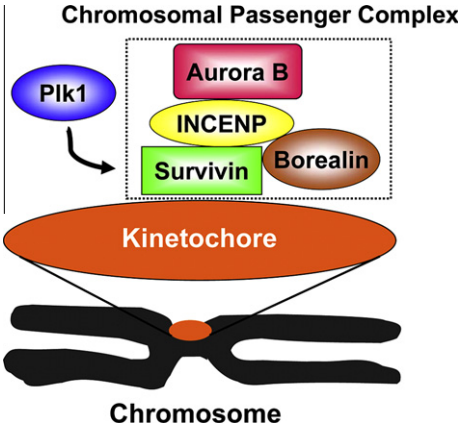

Fig. 4. Diagram of the relationship of chromosomal passenger complex (CPC) and Plk1 in meiosis. Survivin may load to the kinetochores first to provide a platform for the CPC, and regulate the kinetochores localization of Plk1 through a Survivin-Aurora B-Plk1 pathway.

After Survivin depletion, the localization of Plk1 was disrupted, Plk1 failed to accumulate to the kinetochore. This result indicated that the localization of Plk1 to kinetochores needs the involvement of Survivin. And the localization pattern is similar with SAC protein Mad2 after BRCA1 depletion [12]. A recent finding also shows that in Drosophila cells the CPC member Aurora B phosphorylates Polo kinase (Plk1 in mouse) at the centromere in early mitosis, and the phosphorylation requires both INCENP and Aurora B activity [13]. Our data prove that in mammalian meiosis, Surivivin, another member of the CPC is also essential for the proper localization of Plk1 to kinetochores. Due to the fact that Survivin activates Aurora B through mitotic histone H3T3 phosphorylation [14,15], we hypothesize that Survivin may load to the kinetochores first to provide a platform for the CPC, and regulate the kinetochores localization of Plk1 through a Survivin-Aurora B-Plk1 pathway (Fig. 4). The data also provide the evidence that Plk1 involves into the regulation of SAC and set up the relationship between Plk1 and CPC in meiosis.

In conclusion, our results indicate that the CPC member Survivin is a upstream regulator of Plk1 and involves into the localization of Plk1 to kinetochores in mouse oocyte meiosis.

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