Damaged chromatin does not prevent the exit from metaphase I in fused mouse oocytes

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The presence of checkpoint mechanisms which are able to recognize damaged chromatin and thereby prevent exit from metaphase I has been investigated in giant mouse oocytes produced by fusion of a normal metaphase I oocyte with an equivalent oocyte with damaged chromatin. The presence of damaged chromatin did not prevent the onset of anaphase I in both sets of chromatin in the fused cells. Interestingly, fused or unfused cells containing only damaged chromatin failed to enter anaphase and persisted instead in a metaphase-like state. These results demonstrate the fragility of checkpoint controls in mammalian female germ cells.

Key words: checkpoint/chromosomes/oocytes

Introduction

The onset of each cell cycle phase is typically under a strict form of control which ensures that all the events of the preceding phase are completed before entry into the next phase is permitted (Elledge, 1996). Thus, the cell must complete the replication of DNA and the duplication of centrioles before the onset of M-phase (mitosis, meiosis) or the formation of spindle with the proper arrangement of chromosomes before cleavage (Rudner and Murray, 1996). The mechanisms which ensure the completion of these processes are named ‘cell cycle checkpoints’. Whilst these checkpoints are relatively well described in lower organisms and somatic cells, very fragmentary information and conflicting results pertain to germ cells (Fulka Jr et al., 1995a; Soewarto et al., 1995). In these specialized cells the characterization of checkpoints is extremely important, because oocytes and spermatocytes are commonly used for manipulation, i.e. ICSI in humans (Tesarik, 1996) or cloning in farm animals (Fulka Jr et al., 1995). In these experiments was performed in which inhibitor-treated oocytes were fused with controls, and the ability of the resultant giant oocyte to resume meiosis was assessed.

Materials and methods

All reagents were obtained from Sigma (Poole, Dorset, UK). Mouse oocytes were obtained from large antral follicles of pregnant mare’s serum gonadotrophin (PMSG) stimulated females (C57Bl/6J×CBA) injected 44–48 h previously. The oocytes were manipulated in M2 medium: cumulus cells were removed by pipetting and only those oocytes with clearly visible germinal vesicles (GVs) were used for further culture at 37°C in 5% CO2 in air in medium M-199 (Sigma; M-4530) containing Na-pyruvate (0.2 mM), gentamicin (25 µg/ml) and bovine serum albumin (BSA) (3 mg/ml). Under these conditions, germinal vesicle breakdown (GVBD) occurred within 90 min of culture and those oocytes not undergoing GVBD after this time interval were discarded (Fulka Jr and Moor, 1993). The remaining cells were cultured for another 4.5 h and thereafter one half of the cell population was transferred into medium containing etoposide (ETO, 50 µg/ml, topoisomerase II inhibitor) and cultured in this medium for another 2 h. The second half of the cell population was cultured in normal medium. Thereafter, zonae pellucidae were removed from both types of oocyte by pronase treatment (0.5% in phosphate-buffered saline (PBSI) and pairs of oocytes were agglutinated in PBS containing phytohaemagglutinin (PHA, 300 µg/ml). Fusion was then induced by incubation of cells in polyethylene glycol (PEG, Mr. 1000; 0.9 g/ml). PEG was dissolved in pure M-199 and cells were incubated in it for 45–60 s (Fulka Jr et al., 1995b). Thereafter, the oocytes were washed three times in M2 and cultured in M-199. The cells were periodically inspected and after different time intervals they were fixed in acetone, stained in orcein and evaluated under phase contrast microscopy. Alternatively, oocytes were fixed in 4% paraformaldehyde and permeabilized in 0.1% Triton X-100 before an overnight incubation with McAb antibulbin antibody, incubation with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-rat immunoglobulins and staining with propidium iodide. Fused and unfused cells were viewed with a confocal laser scanning microscope (MRC-600, BioRad, Cambridge, UK).
The following combinations of fused cells were prepared:
1) Pairs of mature oocytes, cultured for 8 h, were fused together. Fused partners and unfused single oocytes were cultured thereafter in normal medium for up to 5 h.
2) Fusions were made between pairs of oocytes both containing damaged chromatin. Oocytes were cultured in normal medium for 6 h and for a further 2 h in etoposide-supplemented medium before fusion. As in Study 1, fused and single unfused oocytes were cultured thereafter in normal medium for up to 5 h.
3) Oocytes containing undamaged metaphase spindles (8 h culture in normal medium) were fused with partners containing etoposide-damaged DNA (6 h culture in normal medium plus 2 h culture in etoposide). Giant fused oocytes and unfused oocytes of each type were cultured in normal medium for up to 5 h after fusion.
Each experiment was repeated at least five times.

Results

Group 1

Under our culture conditions, the exit from metaphase I to anaphase–telophase I was observed between 8.5 and 9.5 h after the start of culture. When two metaphase I oocytes matured for 8 h were fused together, the process of maturation continued in the resultant giant cell quite synchronously, i.e. two anaphases, telophases or metaphases II were invariably observed. In no case did we observe two different stages of maturation in any of the fused controls. A total of 95 fused control cells were examined, of which 72 continued meiosis after fusion. The remaining 23 giant controls were arrested in metaphase I (two metaphases I were observed); in these cases, suggesting that an early metaphase I oocyte had probably been fused to a more advanced metaphase I oocyte or that two early metaphase I oocytes had been fused together (Fulka Jr et al., 1995b).

Group 2

When two etoposide-treated oocytes had been fused together, no further meiotic progress in either set of chromatins was ever observed. The 102 giant cells produced in this study were characterized by two heavily condensed sets of chromosomes which each formed a single cluster of chromatin to which the spindle fibres were attached. Even after prolonged periods of culture, two clusters of chromatin were invariably observed in these giant cells; single clusters of chromatin were observed in the unfused oocytes in this group.

Group 3

When we combined an etoposide-treated and an untreated oocyte together, results were quite surprising. In unfused cells (47 pairs evaluated), those oocytes which were not cultured in ETO-supplemented medium continued maturation, whilst the oocytes incubated with ETO remained arrested in a metaphase I-like stage (Figure 2). However, in 150 fused cells, we typically observed the extrusion of two polar bodies. These results indicated that the chromatin exposed to ETO also underwent the transition from metaphase I to a telophase I-like stage. In a further 52 fused giant cells, one normal metaphase I plate together with a chromatin cluster was detected. We analysed this situation in more detail and typically detected in these cells the presence of one normal anaphase to telophase I transition, whilst the second set of chromatin was heavily stretched and the polar body was extruded (Figures 3 and 4). In some cases, this stretched chromatin mass was interrupted whilst in other cases the connection persisted as stretched chromatin connecting the oocyte cytoplasm and the polar body. The results are summarized in Table I.

Discussion

These observations indicate that the checkpoint which regulates progress from metaphase to anaphase in mouse oocytes is ineffective in blocking meiosis in giant cells containing one normal and one damaged set of chromatin. We postulate that the positive stimuli generated by the presence of a normal metaphase spindle is dominant to that emanating from the damaged metaphase chromosomes.

As pointed out by Nicklas (1997), in every cell division the daughter cells must acquire the correct chromosome complement. This is under the control of cell cycle checkpoints
Metaphase checkpoint in mouse oocytes

Figure 3. Fusion of a late metaphase I oocyte with an etoposide-treated oocyte. A normal telophase (arrowheads) can be detected together with heavily extended etoposide-treated chromatin (arrow). Two first polar bodies are extruded (not in same focal plane). Phase contrast, original magnification × 400.

Figure 4. Fusion of a late metaphase I oocyte with an etoposide-treated oocyte. A normal telophase (T) is on the right. Left: staining with anti-tubulin antibody; right: staining with propidium iodide (original magnification × 300).

Table I. The onset of anaphase-telophase in fused maturing oocytes

<table>
<thead>
<tr>
<th>Combination of fusion</th>
<th>No. of oocytes fused</th>
<th>Stage of maturation in fused cells</th>
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<tbody>
<tr>
<td>MI × MI</td>
<td>95</td>
<td>2×A-T transition (72 cells)</td>
</tr>
<tr>
<td>MI-ETO × MI-ETO</td>
<td>102</td>
<td>2×MI (23 cells)</td>
</tr>
<tr>
<td>MI × MI-ETO</td>
<td>202</td>
<td>2×MI-ETO (two clusters, 102 cells)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2×A-T transition (150 cells)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2×MI + chromatin cluster (52 cells)</td>
</tr>
</tbody>
</table>

MI = normal metaphase I oocyte, MI-ETO = etoposide-treated metaphase I oocyte, A-T = transition from metaphase to anaphase-telophase I.

which ensure that, for example, the events of DNA replication and chromosome segregation are completed with high fidelity (Elledge, 1996). It is generally accepted, but not adequately proved, that oocytes and early embryonic cells lack these mechanisms (Hartwell and Weinert, 1989). Thus, in humans, embryonic mortality is extremely high. As discussed by Handyside (1996), the frequency of mosaicism in human preimplantation embryos indicates many irregularities in the processes of meiotic maturation, fertilization and postzygotic mitotic divisions. This high rate of embryonic mortality probably indicates the lack of immutable checkpoints in germ cells.

These theoretical conclusions are supported by experiments which show, for example, that the immature mouse GV staged oocyte is unable to recognize replicating DNA which is introduced into it in the form of an S-phase nucleus (Fulka Jr et al., 1995b). On the other hand, we have previously demonstrated that the fusion of an early metaphase I oocyte to a late metaphase I oocyte prevents the progress of maturation (Fulka Jr et al., 1995). This clearly indicates the existence of a checkpoint mechanism which is able to recognize the less advanced metaphase. Thus, it is surprising that chromatin which is clearly severely damaged is apparently unable to prevent anaphase onset.

One possible explanation may be the equilibration of different cytoplasmic factors which are involved in the regulation of oocyte maturation. The levels of maturation promoting factor (MPF, expressed as H1 kinase activity) are identical in ETO treated and in normally matured oocytes (Kalab et al., unpublished data). Moreover, we were able to detect a drop in H1 kinase activity during the transition from metaphase to anaphase in combinations 1 and 3 (data not shown). As showed by Hampl and Eppig (1995), the levels of H1 kinase rise slowly during maturation and are much lower in oocytes matured for 6 h compared to oocytes cultured for 8 h. Thus it is possible that in Study 1, in the case of the 23 giant oocytes with two MI, the oocyte with the lower level of H1 kinase decreased the level of this kinase in the more advanced oocyte. Hence, the expected anaphase onset in giant cells is prevented. However, other factors are also involved in the regulation of anaphase onset, i.e. various centromeric proteins involved in the spindle-kinetochore attachment (Rudner and Murray, 1996).

The limited fidelity of mammalian oocytes to chromosomal errors has also been recently demonstrated by Pyrzynska et al. (1996), who showed that oocytes fertilized at the GV stage continued maturation and reached metaphase II. In this situation the fertilizing spermatozoa remained condensed throughout the whole process of maturation, resembling in fact the situation present in our fused cells. Taken together, we believe that our results demonstrate the limited sensitivity of mammalian germ cells to certain errors. A greater degree of sensitivity is probably established at a much later stage and leads to the elimination of abnormal embryos.

Acknowledgements

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References


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