

Deficiency of DNA fragmentation factor 45 results in reduced oocyte apoptosis in response to doxorubicin

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Dear Editor:

Apoptosis plays a prominent role in ovarian development and function [1-4]. During follicle development, the vast majority of follicles undergo atresia as a consequence of apoptosis of constituent oocyte or follicular cells, or both, failing to complete the maturation process. Atresia occurs at all stages of follicular development during the growth and development of follicles. Atresia of the primordial and primary follicles is initiated by oocyte apoptosis, followed by the death of the granulosa cells [1-4]. In comparison, the reduction of the large number of growing follicles to a single ovulatory follicle is achieved primarily by the cell death of granulosa cells [1-4]. Members of the Bcl-2 and caspase families play important roles in the regulation of ovarian cell death, both in follicle development and in response to chemotherapeutic agents [5].

DNA fragmentation factor (DFF), consisting of DFF40 (also called caspase-activated Dnase (CAD)) and DFF45 (also called inhibitor of caspase-activated Dnase (ICAD)), cleaves DNA into nucleosomal-sized fragments during apoptosis [6]. To understand the *in vivo* function of DFF45, we previously generated DFF45 mutant mice [7, 8]. We found that DFF45-deficient cells are more resistant to apoptosis than wild-type control cells after exposure to several apoptotic stimuli [7-9]. DFF45 mutant mice exhibit enhanced learning and memory [9, 10]. Collectively, these data indicated a critical role of DFF45 in normal apoptosis and tissue homeostasis.

To examine whether DFF45 gene disruption affects

oocyte apoptosis, we collected oocytes from wild-type or *DFF45*^{-/-} females. The oocytes were then denuded and cultured with or without 200 nM of the anti-cancer drug, doxorubicin (DXR), to induce apoptosis.

The superovulation rate of *DFF45*^{-/-} females was comparable with that of wild-type females (18.00 ± 2.69 vs. 16.63 ± 3.42 oocytes/mouse, respectively; mean ± SEM; n=8 mice/genotype). Although no significant difference (*p* > 0.05) was found in spontaneous apoptosis in oocytes retrieved from wild-type vs *DFF45*^{-/-} female mice (Figure 1A and 1B), the incidence of DXR-induced apoptosis was significantly lower in oocytes harvested from *DFF45*^{-/-} female mice than that from wild-type sisters, when treated in parallel with DXR for 20 h. By 48 h in culture, apoptosis, either spontaneous or drug-induced, was similar in oocytes harvested from wild-type and *DFF45*^{-/-} females (Figure 1B).

We analyzed serial ovarian sections from wild-type and *DFF45*^{-/-} female mice to examine the potential effect of the loss of DFF45 on follicle development. At 18 months of age, we found that *DFF45*^{-/-} females possess more numbers of both non-atretic secondary follicle and graafian follicle than wild-type females (Supplementary Figure 1). Ovaries of wild-type mice contained follicles with many TUNEL-positive cells (Supplementary Figure 1B and 1C). The number of TUNEL-positive cells per follicle was significantly lower (*p* < 0.05) in *DFF45*^{-/-} mice (25.50 ± 5.00), compared with follicles in wild-type mice (53.80 ± 10.63).

In 4-day-old females, we found no significant difference in the number of primordial and primary follicles between wild-type and *DFF45*^{-/-} females (Supplementary Figure 2A), suggesting that follicle endowment in wild-type and *DFF45*^{-/-} female mice shortly after birth were comparable. At 14 days of age, the number of late primary follicles was slightly increased in *DFF45*^{-/-} females compared with that in wild-type females (Supplementary Figure 2B). At

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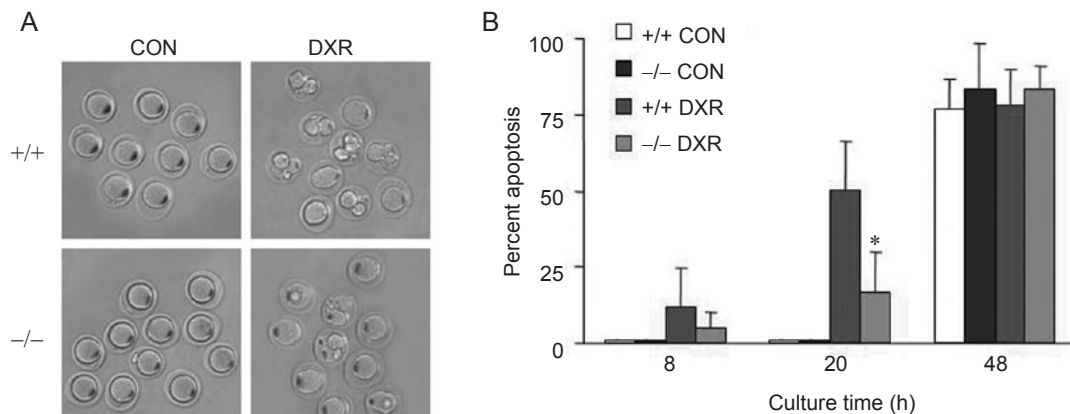


Figure 1 *DFF45*^{-/-} oocytes exhibit decreased DXR-induced cell death compared with wild-type oocytes. Oocytes collected from wild-type (+/+) and *DFF45*-deficient (-/-) female mice were cultured *in vitro* in the absence or presence of 200 nM DXR for 8, 20, and 48 h. At the termination of culture, the occurrence of apoptosis was assessed first by observation of nuclear fragmentation and then by DAPI staining. **(A)** Representative images capturing the cellular morphology in pools of wild-type and *DFF45*-deficient oocytes cultured without DXR for 20 h (CON); and pools of wild-type and *DFF45*-deficient oocytes cultured with 200 nM DXR for 20 h (DXR). **(B)** Bar graph of occurrence of apoptosis in oocytes from wild-type and *DFF45*-deficient mice after *in vitro* culture in the absence (CON) or in the presence of DXR (DXR) for 8, 20, and 48 h. The experiments were repeated with *n* = 3 families for each genotype. Data are given as mean + SEM. **p* < 0.05. Student's *t*-test is used for comparison between genotypes for the same treatment.

42 days of age, *DFF45*^{-/-} female mice possessed fewer early primary follicles (Supplementary Figure 2C) compared with those in wild-type counterparts. Overall, *DFF45*^{-/-} mice do not exhibit overt changes in follicle numbers at the age groups we examined, despite their having reduced number of TUNEL-positive cells (Supplementary Figure 2D-2F). One likely possibility is that other nucleases are more important for developmental cell death in the ovary. Another possibility is that nucleases are not required for developmental cell death in the ovary.

The ovarian volumes in the wild-type and *DFF45*^{-/-} female mice (*n* = 10, each) at 4 days, 14 days, 42 days, and 18 months of age are not significantly different between genotypes (*p* > 0.05) (data not shown). In conclusion, this study demonstrated the functional importance of *DFF45* in execution of apoptosis in cultured oocytes in response to chemotherapeutic agents, but *DFF45* is not critically required for ovarian follicular cell maturation and development.

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(Supplementary Information is linked to the online version of the paper on the Cell Research website.)