

Morphological Anomaly of Primordial Follicle in γ -Irradiated Mice

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Abstract

Ovarian follicles are faced with one of two fates, atresia or development. Up to 99% of follicles become degenerated rather than ovulated in female life span. Thus, atresia occurs at all stages of follicle development in mammalian ovaries. In the present experiment, the effect of γ -radiation on primordial follicles was morphologically analyzed in a mouse ovary. Thirty-seven percent of the primordial follicles in the non-irradiated control mice ovaries were abnormal. At day 8 post irradiation, most of primordial follicles became atretic. They lost their integrity of architecture in the follicular shape. Then, all the oocytes disappeared from the follicles. And only 3 to 4 granulosa cells lay down onto the basement membrane. Disappearance of granulosa cells or oocytes resulted from the radiation-induced apoptotic process. It is definitely clear that γ -radiation induces rapid apoptotic degeneration of the primordial follicles. The morphological degeneration induced by radiation in the primordial follicles can be used as an experimental model to draw out a deeper insight for radioprotectant researches.

Key Words : radiation, primary follicle, degeneration, mouse

1. Introduction

Most follicles remain in the resting pool of nongrowing primordial follicles [1]. Follicular activation is the transition of a follicle from the inactive primordial follicle stage to the active primary follicle stage [2]. The flattened granulosa

cells lining the growing follicles acquire receptors for follicle stimulating hormone (FSH) and proliferate to form two or three layers in secondary follicles [3, 4]. Atresia occurs at all stages of follicle development [5]. Two major stages of cell degeneration can be distinguished in the mammalian ovary: degeneration of germ cells,

defined as attrition, which accounts for the main loss of oocytes [6] and occurs, in specific, prenatally, and follicle degeneration, defined as atresia, which occurs during postnatal reproductive life [7].

Apoptosis, a regulated form of cell death, is a physiological process essential for the normal tissue homeostasis [7] in the absence of immune surveillance [8]. Ovarian follicular degeneration or atresia is a hormonally controlled apoptotic process, whereby degenerating follicles are eliminated in a coordinated fashion [9]. The establishment of a set number of primordial follicles in the peri-natal ovary is believed to determine the functional reproductive life span of females as pathological conditions which accelerate the depletion of the follicle pool lead to premature reproductive acyclicity and senescence [10]. One of such pathologic stimuli that could accelerate the follicular atresia was radiation [11]. In both normal tissues and tumors apoptosis not only occurs spontaneously but can be induced by irradiation [12]. It was reported that primordial oocytes of rats and mice were more sensitive to radiation than oocytes in the growing follicles [13]. Radiation induced cell apoptosis [12] and impaired the ovarian functions [14]. Mice irradiated at three weeks of age showed the maximum radiation effect on the ovaries [15].

Although there are many reports concerning oocyte degeneration after irradiation, little has been known on the morphologic characteristics of primordial follicles. Especially the morphological changes of granulosa cells are thought to play a focal role in follicular degeneration. Therefore, in the present experiment, morphologic analyses were performed to clarify the effect of γ -radiation on primordial follicles in the mouse ovary.

2. Materials and Methods

According to Lee et al. [16], immature female

mice (ICR strain, 3 week-old) were whole body irradiated with γ -radiation (^{60}Co , dose rate: 0.5Gy/min., source strength: approximately $1.5 \times 10^{14}\text{Bq}$, Panoramic Irradiator, Atomic Energy of Canada Ltd.) at Korea Atomic Energy Research Institute. The dose of radiation was $\text{LD}_{80(30)}$ (8.3Gy), with which 80% of mice irradiated were dead within 30 days post irradiation. The dose rate was chosen for the induction of massive atresia of follicles in the ovary. The ovaries were collected at 0h, 6h, 12h, 1d, 2d, 4d, and 8d post irradiation from irradiated and non-irradiated control animals. The number of individuals in each experimental group was five. To observe the changes in the architecture of the primordial follicles, post fixation using 1% of osmium tetroxide (Sigma Chem. Co. MO) was conducted for 2h at 4°C after prefixation with 2.5% glutaraldehyde/0.1M phosphate buffer (pH 7.3). The embedding of specimens after alcoholic dehydration and displacement by propylene oxide was carried out in an epon mixture [Poly/Bed 812 resin (Epon 812) : Dodecenylsuccinic Anhydride : Nadic Methyl Anhydride : 2, 4, 6-tri (dimethylaminomethyl) phenol (DMP-30) = 19.3 : 12.3 : 9.4 : 0.6 ml, Polysciences Inc.]. Using ultra-microtome (Leica), semithin sectionates were prepared with 1 μm in thickness and stained with 1% toluidine blue O in 1% borax solution. The largest cross sectionates were used in this study. Observation of morphological changes was done under a light microscope (Olympus).

3. Results and Discussion

3.1. Results

About 37% of the primordial follicles in non-irradiated control mouse ovaries were degenerating. In case of normal non-irradiated primordial follicles, flattened granulosa cells

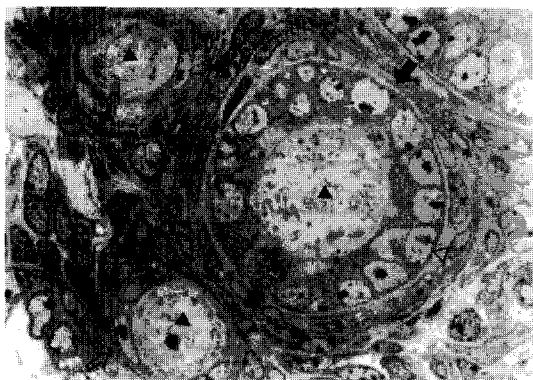


Fig. 1. Microphotograph of Normal Primordial and Primary Follicles in the Mouse Ovary. Normal Primordial (open arrows) and Primary (large black arrow) Follicles were Clearly Shown. Arrow Heads Indicate Oocytes. Mitotic Granulosa Cell was Shown in Primary Follicle (small black arrow). Clear Basement Membrane in Primary Follicle was Identified. Original Magnification: x 1,000.



Fig. 2. Microphotograph of Primordial and Primary Follicles in the Irradiated Mouse Ovary. At 6 Hours After γ -Irradiation, the Oocyte of Primordial Follicle was Degenerated by Means of Apoptosis (large open arrow). The Oocyte Disappeared from the Degenerating Primordial Follicle (small open arrow). Apoptotic Granulosa Cell (small black arrow) in Primary Follicle (large black arrow) was Shown. Note that the Nuclear Membrane of Oocyte was Shrunk. Original Magnification: x 1,000.

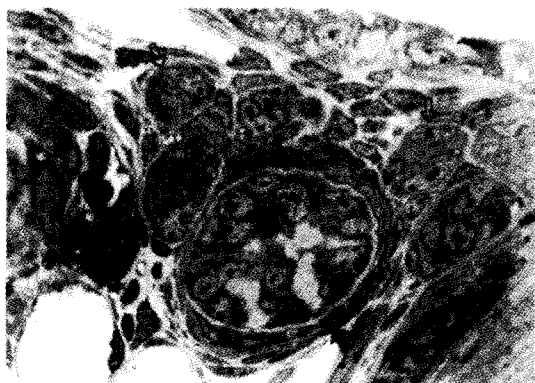


Fig. 3. Microphotograph of Atretic Primordial and Primary Follicles in the Irradiated Mouse Ovary. Four Days after γ -Irradiation, Irregular and Malformed Granulosa Cells were Shown in the Primordial (open arrows) and Primary Follicles (black arrow) in the Irradiated Mouse Ovary. All the Oocytes Disappeared. Only the Follicle-like Structures Could be Seen. Original Magnification: x 1,000.

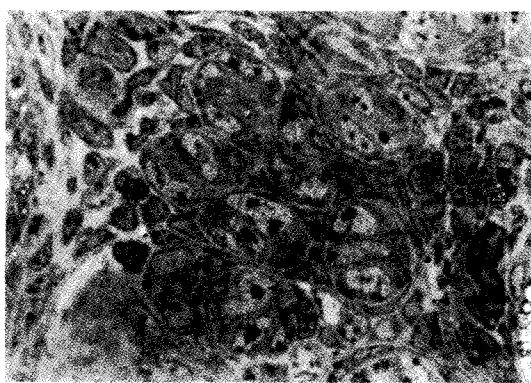


Fig. 4. Microphotograph of Degenerating Primordial Follicles. Eight Days after γ -Irradiation, the Follicle-like Structures were Only Seen and All the Oocytes Disappeared from the Follicle. Granulosa Cells were Malformed and Only Three or Four Granulosa Cells Lay Down Onto the Basement Membrane (among them two primordial follicles were indicated by open arrows). Original Magnification: x 1,000.

surrounded the rounded oocytes. There was a clear and even basement membrane in both primary and primordial follicles (Fig 1). The initial atretic characteristics of primordial follicles were apoptosis of granulosa cells surrounding intact oocytes, apoptosis of oocytes with healthy granulosa cells, and apoptosis of oocytes with malformed granulosa cells (Fig. 2).

In a normal primary follicle, mitotic granulosa cell was shown (Fig. 1). At 6h post-irradiation, some of primordial follicles were degenerated. This degeneration was envisaged by nuclear condensation and margination which were characteristic of cell apoptosis. The granulosa cells in primary follicles and the oocytes in primordial follicles were on the way of apoptosis (Fig. 2). At 4d post irradiation, some of granulosa cells disappeared from primary follicles. Most of the primordial oocytes disappeared as well (Fig. 3). At 8d post irradiation, peculiar abnormal primordial follicles became abundant. Three or four abnormal granulosa cells were only scored in primordial the follicle-like structures (Fig. 4).

3.2. Discussion

The depletion of primordial follicles can occur as a consequence of either oocyte attrition or initiation of growth [17]. During the onset of follicle growth, the flattened granulosa cells of primordial follicles become cuboidal and begin to proliferate, and the enclosed oocyte begins to grow [5]. In the present results, some of granulosa cells in growing primary follicles showed mitotic activities. This apparently indicates the initiation phase of primary follicles. The average mitotic activity of granulosa cells of healthy follicles is more closely related to the follicular granulosa mass than to the particular phase of the ovarian cycle [18]. It has been reported that follicular activation is probably not dependent on

gonadotropic hormones [19]. And FSH does not rescue radiation-induced atresia in mouse ovary [20].

The criteria by which apoptosis is characterized include the loss of cell volume accompanied by nuclear pyknosis resulting from the margination of the chromatin and its redistribution against the nuclear envelope [21]. Morphologic patterns of follicular degeneration give a new insight of ovarian tissue dynamics and new information in an ovary with a continuous phenotypic transformation of its cellular components.

In the present experiment, we clearly identified the radiation-induced apoptosis of oocyte in primordial follicles. Thirty-seven percent of the primordial follicles were abnormal in normal immature mouse ovary. But at 8d post irradiation, there were no more primordial follicles which were morphologically normal. The shapes of these follicles had follicle-like structures without oocytes which presumably disappeared after irradiation. Ratts et al. (1995)[22] observed numerous aberrantly formed primordial follicle-like structures, resembling "donuts", containing a single layer of granulosa cells without an oocyte in *bcl₂* deficient transgenic mouse ovary. These follicles might be in the same structure as the apoptotic primordial follicles in the present experiment. It was thought that these might exist slowly degenerating follicles and that the degenerating follicles identified in the present experiment steeply became abnormal. Thus, *bcl₂* deficient, but irradiated, mouse ovarian follicles became progressively atretic. Kim et al. [11, 20] reported that follicular atresia induced by γ -radiation was accelerated via an apoptosis of granulosa cells. Radiation triggers acute anomalies of primordial follicles. Its molecular mechanism remains to be further elucidated. Granulosa cells of preantral and antral follicles were more radiosensitive than oocytes [20]. In the present

results, oocytes of primordial follicles experienced a rapid apoptotic degeneration after irradiation. The shape of the basement membrane maintained relatively normal in the atretic follicles.

It is concluded that the ionizing radiation has an acute detrimental effect on primordial follicles. Further information essential for the radioprotectant study can be derived out from the morphological changes of primordial follicles by radiation.

4. Conclusions

Radiation induced acute and steep degeneration of primordial follicles in the mouse ovary. Various anomalies in the primordial follicles occurred during 6 hours after irradiation. The characteristics of follicular atresia induced by γ -radiation includes apoptosis of oocyte and of granulosa cells, as well. In the primary follicles, apoptosis, in general, occurs in granulosa cells. With time after irradiation, primordial follicles became abnormal. Within the basement membrane, granulosa cells could only be scored after irradiation. Granulosa cells abnormally proliferated or disappeared, thereafter. It was confirmed that the collapse of the follicular architecture, i.e. disappearance of oocytes or granulosa cells and abnormal proliferation of granulosa cells, was triggered by γ -radiation. The morphological degeneration induced by radiation in the primordial follicles can be used as an experimental model to draw out a deeper insight for radioprotectant researches.

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