Gamma-irradiation increased meiotic crossovers in mouse spermatocytes

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In mice, the occurrence of immunofluorescent foci for mismatch repair protein MLH1 correlates closely with the occurrence of crossovers, as detected genetically, and MLH1 foci represent virtually all prospective crossover positions. To examine the effects of γ-irradiation on meiotic crossovers in mouse spermatocytes, male mice were subjected to whole-body γ-irradiation at different sub-stages of meiotic prophase and crossovers on synaptonemal complexes (SCs) were analysed by visualising and quantifying the immunofluorescent MLH1 foci. At both 24 and 48 h after exposure, significant dose-dependent increases in the number of total MLH1 foci per spermatocyte were observed at late zygotene–early pachytene with the gradient increase of radiation dose from 0, 1.5, 3–6 Gy. Furthermore, irradiation at preleptotene–leptotene still led to significant dose-dependent increased meiotic crossovers in the spermatocytes analysed 120 h after exposure. In further analysis, these dose-dependent increases in the number of total MLH1 foci per cell were attributed to significant dose-dependent decreases in autosomal SCs with 0 MLH1 foci, and the dose-dependent increases in autosomal SCs with 2 MLH1 foci and the percentage of cells with MLH1 focus on XY bivalent. The increased number of cells with an MLH1 focus on the pseudoautosomal regions (PARs) may indicate that there is a delay in meiotic progression in the irradiated cells. Although significant dose-dependent increases in the number of total MLH1 foci per cell were examined 24, 48 or 120 h after exposure with the gradient increase of radiation doses, these increases were mild compared to the control groups. This suggests that there is tight control of crossover formation (at least with respect to MLH1 foci number). The mechanisms underlying irradiation-induced DNA lesion repair, cellular responses independent of DNA damage and meiotic crossover homeostasis in mammals will be the subjects of future study.

Introduction

Meiotic crossovers are generated by homologous recombination initiated by DNA double-strand breaks (DSBs) formed by the topoisomerase-like SPO11 protein (1). There are more DSBs than crossovers, in some cases, substantially more (>10-fold), and DSBs that do not become crossovers are repaired to give noncrossovers instead (2,3). Meiotic crossovers tend to be maintained at the expense of noncrossovers when DSBs were reduced in yeast spo11 hypomorphs and genomic loci differ in expression of the ‘crossover homeostasis’ (3). Moreover, analysis of the correlation between crossovers and noncrossovers using genome-wide DNA microarrays has provided further support for crossover homeostasis in Saccharomyces cerevisiae (4). Nevertheless, crossover homeostasis was part of normal crossover control in wild type and reduced in zip2 and zip4 mutants. Another mechanism of crossover control named crossover invariance is found in fission yeast, in which the repair of DSBs at strong hotspots is predominated by intersister Holliday junction formation without DNA DSBs (5). Ionising radiation results in a significant 2.7- to 3.6-fold increase in germine expanded simple tandem repeat (ESTR) mutation rate in male mice mated 4, 5 and 6 weeks after exposure but had no effect on meiotic recombination frequencies assayed on six chromosomes using 25 polymorphic microsatellite loci covering 421 cM of the mouse genome (6). Therefore, it is uncertain whether meiotic recombination plays a role in mutation at mouse ESTR loci and whether this mutation arises from a genome-wide increase in meiotic recombination frequencies in exposed animals. During meiotic prophase, DNA repair is competent in spermatocyte nuclei and at the leptotene stage, an endogenous DSBs are introduced by SPO11 protein to provoke homologous recombination and converted into crossovers (13–15). As expected, irradiation-induced extra DSBs at the leptotene stage can also be converted into crossovers and lead to a significant increase in meiotic recombination frequency in mouse spermatocytes (16). To obtain more insight into the effects of irradiation on meiotic recombination, we irradiated male mice at different sub-stages of meiotic prophase in vivo to examine whether this would change the frequency of meiotic crossovers in spermatocytes.

Fortunately, current immunofluorescence techniques make the analysis of meiotic recombination across the whole genome more precise and the visualisation of some important meiotic

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structures on synaptosomal complexes (SCs) more distinguishable. In this immunocytoanalytical analysis, antibodies against SYCP1 (SC protein 1; marks transverse elements of the SC), SYCP3 (marks lateral elements of the SC), MLH1 (an ortholog of the Escherichia coli Mut L mismatch repair protein; marks recombination sites along the SC), and CREST (calcinosism, Raynaud’s phenomenon, esophageal dysfunction, sclerodactyly, telangiectasia; marks the centromere) were used to identify meiotic recombination patterns and fidelity of chromosome pairing in pachynema stage cells (17–19). Most importantly, the DNA mismatch repair protein MLH1 accumulates at sites of crossing-over during pachynema and forms distinct foci localised to chiasmata precociously induced by okadaic acid (20–22). The antibodies against MLH1 identify the number and location of meiotic crossovers on SCs in both mouse (20,21) and human spermatocytes (18,23,24). In mice, the occurrence of immunofluorescent MLH1 foci correlated closely with the occurrence of crossovers as detected genetically (17,25), which implies that MLH1 foci represent nearly all prospective crossover positions in this species. Here, we took advantage of this immunocytoanalytical analysis and induced DNA damage by whole-body γ-irradiation of male mice to examine whether irradiation at different sub-stages of meiotic prophase will change the meiotic crossovers in spermatocyte nuclei.

Materials and methods

Mice

Thirty-six adult male C57BL/6Slac mice were obtained from a single dedicated supply colony at Slac laboratory Animal (Shanghai, China), ranked by body weight and randomly assigned to irradiation (27 mice) and control groups (9 mice). All the mice were housed in an air-conditioned animal facility with a 12-h light cycle.

Sub-stage-specific radiation treatment of spermatocyte during meiotic prophase

Mice were placed in a carton box with a lucite top and irradiated with doses of 1.5, 3 and 6 Gy of γCo-irradiation at a dose rate of 0.5546 Gy min⁻¹ from an FCC-700 Concentric Rotary ⁶⁰Co Therapeutic Apparatus (the First Affiliated Hospital of Anhui Medical University, Anhui, China). Stage-specific radiation treatments were accomplished by varying spermatocyte harvest times following irradiation. Times chosen are consistent with both the spermatogenic cell cycle and the maturation sequence (16,26) and are schematically represented in Figure 1. Spermatocytes harvested after 24 or 48 h for meiotic recombination analysis on SC (at mid-pachynema, approximately Stages IV–VIII of the cycle of the seminiferous epithelium) were considered to reveal the effects of irradiation at late zygote–early pachynema and those analysed after 120 h reveal the treatment specifically at preleptotene–leptotene (16,26). The mice exposed to different doses of γ-irradiation were fed and housed until sacrificed to obtain epididymides and testes for meiotic recombination analysis on SC at 24, 48 and 120 h after irradiation, which correspond to the stage of late zygote–early pachynema, late zygote–early pachynema and preleptotene–leptotene within meiotic prophase, respectively. At each stage of meiotic prophase, nine male mice were evenly divided into three groups and exposed to 1.5, 3 and 6 Gy of γ-irradiation, respectively.

TO BE CONCLUDED

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Table I. The frequency of MLH1 foci distributed on SCs in pachytene spermatocyte nuclei exposed to different doses of {\gamma}-radiation at late zygotene–early pachytene (analysed 24 h after exposure)

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>Number of mice analysed</th>
<th>Number of cells</th>
<th>Total MLH1 foci per cell (mean ± SD)</th>
<th>Autosomal SCs with different number of MLH1 foci (mean/cell)</th>
<th>Unsynapsed XY (mean; %cells)</th>
<th>XY with MLH1 focus (mean; %cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>103</td>
<td>22.48 ± 2.81</td>
<td>0.67</td>
<td>14.57</td>
<td>3.73</td>
</tr>
<tr>
<td>1.5</td>
<td>3</td>
<td>112</td>
<td>23.28 ± 2.21</td>
<td>0.29</td>
<td>14.53</td>
<td>4.14</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>96</td>
<td>23.90 ± 2.14</td>
<td>0.15*</td>
<td>14.18</td>
<td>4.60*</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>90</td>
<td>24.33 ± 2.03*</td>
<td>0.01*</td>
<td>14.31</td>
<td>4.62*</td>
</tr>
</tbody>
</table>

*Refers to highly significant difference (P < 0.01) between a tested and the control group.

**Refers to significant difference (P ≤ 0.05) between a tested and the control group.

Fig. 2. Increased meiotic recombination in the spermatocytes irradiated at late zygotene–early pachytene (analysed 24 h after exposure). (A) Immunofluorescent staining for MLH1 (green), SCP3 (red) and CREST (blue) SCs in mid-pachytene nuclei from the control and irritated mice. Sex chromosomes are marked by X and Y. The asterisk indicates unsynapsed XY body, arrows indicate XY body with MLH1 foci. (a) Mid-pachytene nuclei exposed in 0 Gy (control) of {\gamma}-radiation and the number of MLH1 foci is 22. (b) Mid-pachytene nuclei exposed in 1.5 Gy of {\gamma}-radiation and the total MLH1 foci is 23. (c) Mid-pachytene nuclei exposed in 3 Gy of {\gamma}-radiation and the total MLH1 foci is 24. (d) Mid-pachytene nuclei exposed in 6 Gy of {\gamma}-radiation and the total MLH1 foci is 29. (B) Graph of the average number of MLH1 foci in mid-pachytene nuclei from the control (=22.48, with n = 103 nuclei per three mice) and irritated mice exposed in 1.5 Gy (=23.28, with n = 112 nuclei per three mice), 3 Gy (=23.90, with n = 96 nuclei per three mice) and 6 Gy (=24.33, with n = 90 nuclei per three mice) of {\gamma}-radiation. Asterisk indicates a significant difference (P = 0.03 and P < 0.01 for 3 and 6 Gy cohort, respectively; independent sample T-test) as compared to the control. Error bars represent the standard error of the mean.
number of autosomal SCs with 0 MLH1 focus per cell exhibited a tendency to decrease in the nuclei exposed to 1.5 Gy (0.29; \( P = 0.08 \)), 3 Gy (0.15; \( P < 0.01 \)) and 6 Gy (0.01; \( P < 0.01 \)) of \( \gamma \)-radiation. Accordingly, the mean number of autosomal SCs with 2 MLH1 foci showed a tendency to increase in the cells exposed to 1.5 Gy (4.14; \( P = 0.50 \)), 3 Gy (4.60; \( P = 0.04 \)) and 6 Gy (4.62; \( P < 0.01 \)) of \( \gamma \)-radiation compared to the control (3.73).

Forty-eight hours after exposure, significant dose-dependent increases in the mean number of MLH1 foci per cell were found in the spermatocytes exposed to 1.5 Gy (23.81 ± 1.90; \( P < 0.01 \)), 3 Gy (23.85 ± 2.02; \( P < 0.01 \)) and 6 Gy (24.41 ± 1.97; \( P < 0.01 \)) of \( \gamma \)-radiation compared with the control group (22.65 ± 2.18) (Table II). For further analysis, we also grouped autosomal SCs in those with 0, 1, 2 and 3 MLH1 foci (Table II).

The mean number of autosomal SCs with 0 MLH1 focus per cell showed significant dose-related decreases in the nuclei exposed to 1.5 Gy (0.05; \( P < 0.01 \)), 3 Gy (0.03; \( P < 0.01 \)) and 6 Gy (0.04; \( P < 0.01 \)) of \( \gamma \)-radiation compared to the control (0.26).

Correspondingly, significantly dose-dependent increases of autosomal SCs with 2 MLH1 foci were observed in the cells exposed to 1.5 Gy (4.32; \( P = 0.01 \)), 3 Gy (4.32; \( P = 0.01 \)) and 6 Gy (4.91; \( P < 0.01 \)) of \( \gamma \)-radiation compared to the control (3.63). Meanwhile, compared to the control (27.36), the percentage of cells with MLH1 focus on XY bivalent also exhibited dose-dependent increases from 46.97 (\( P < 0.01 \)) to 50.56 (\( P < 0.01 \)) in the nuclei exposed to 1.5, 3 and 6 Gy of \( \gamma \)-radiation, respectively (Table II).

We analysed 430 pachytene stage cells which were considered to reveal the effects of \( \gamma \)-radiation on spermatocytes at preleptotene–leptotene of prophase (analysed 120 h after exposure). Compared with the control group (22.66 ± 2.13), significant dose-dependent increases were found in the mean of MLH1 foci per cell in spermatocytes exposed to 1.5 Gy (23.94 ± 1.95; \( P < 0.01 \)), 3 Gy (23.97 ± 1.82; \( P < 0.01 \)) and 6 Gy (24.06 ± 1.63; \( P < 0.01 \)) of \( \gamma \)-radiation (Table III). We also grouped SCs in those with 0, 1, 2 and 3 MLH1 foci for further analysis (Table III).

Compared to the control group, significant dose-related decreases in the number of autosomal SCs with 0 MLH1 focus were observed in the spermatocytes exposed to 1.5 Gy (\( P < 0.01 \)), 3 Gy (\( P = 0.01 \)) and 6 Gy (\( P < 0.01 \)) of \( \gamma \)-radiation, while significant dose-dependent increases in the number of autosomal SCs with 2 MLH1 foci were seen in the cells exposed to different doses of \( \gamma \)-radiation (1.5 Gy, \( P = 0.05 \); 3 Gy, \( P = 0.02 \); 6 Gy, \( P = 0.02 \)). Additionally, the percentage of cells with MLH1 focus on XY bivalent also exhibited dose-related increases in the cells exposed to different doses of \( \gamma \)-radiation compared to the control group (\( P < 0.01 \)) (Table III).

**Discussion**

To visualise and quantify the number of meiotic crossovers by immunofluorescent staining, we employed the antibody detecting MLH1 foci, which mark almost all crossover sites in the mouse along the SCs (17,25,27). The average number of MLH1 foci per spermatocyte of three control groups (exposed to 0 Gy of \( \gamma \) radiation) is 22.48 (Figure 2A; Table I), 22.65 (Table II) and 22.66 (Table III), respectively, which are within the range of the average number of crossover frequencies per cell observed for C57BL (22.70) and other mouse strains (22.6–23.9) (21,28,29). Most autosomal SCs in control group have 1 or 2 MLH1 foci, but few autosomal SCs with 3 or 0 foci were observed (Tables I–III). As exhibited in Figure 2A, the shorter SCs usually have 1 MLH1 focus, while the longer SCs average more than 1 focus, which is consistent with previous studies that have reported a positive correlation between chromosome length and number of crossovers (21,30).

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**Table II.** The frequency of MLH1 foci distributed on SCs in pachytene spermatocyte nuclei exposed to different doses of \( \gamma \)-radiation at late zygotene–early pachytene (analysed 48 h after exposure)

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>Number of mice</th>
<th>Number of cells analysed</th>
<th>Total MLH1 foci per cell (mean ± SD)</th>
<th>Autosomal SCs with different number of MLH1 foci (mean/cell)</th>
<th>Others</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3</td>
<td>107</td>
<td>22.65 ± 2.18</td>
<td>0.26</td>
<td>25.15</td>
<td>3.63</td>
<td>0.01</td>
<td>0.00</td>
<td>27.36</td>
</tr>
<tr>
<td>1.5</td>
<td>3</td>
<td>133</td>
<td>23.81 ± 1.90</td>
<td>0.05</td>
<td>14.61</td>
<td>4.32</td>
<td>0.03</td>
<td>4.55</td>
<td>46.97</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>100</td>
<td>23.85 ± 2.02</td>
<td>0.03</td>
<td>14.62</td>
<td>4.32</td>
<td>0.03</td>
<td>4.00</td>
<td>49.00</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>90</td>
<td>24.41 ± 1.97</td>
<td>0.04</td>
<td>13.99</td>
<td>4.91</td>
<td>0.03</td>
<td>2.25</td>
<td>50.56</td>
</tr>
</tbody>
</table>

*Refers to highly significant difference (\( P \leq 0.01 \)) between a tested and the control group.

**Table III.** The frequency of MLH1 foci distributed on SCs in pachytene spermatocyte nuclei exposed to different doses of \( \gamma \)-radiation at preleptotene–leptotene of prophase (analysed 120 h after exposure)

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>Number of mice</th>
<th>Number of cells</th>
<th>Total MLH1 foci per cell (mean ± SD)</th>
<th>Autosomal SCs with different number of MLH1 foci (mean/cell)</th>
<th>Others</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3</td>
<td>99</td>
<td>22.66 ± 2.13</td>
<td>0.58</td>
<td>14.39</td>
<td>4.03</td>
<td>0.00</td>
<td>7.14</td>
<td>19.39</td>
</tr>
<tr>
<td>1.5</td>
<td>3</td>
<td>106</td>
<td>23.94 ± 1.95</td>
<td>0.07</td>
<td>14.37</td>
<td>4.51</td>
<td>0.05</td>
<td>9.52</td>
<td>40.00</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>121</td>
<td>23.97 ± 1.82</td>
<td>0.09</td>
<td>14.33</td>
<td>4.55</td>
<td>0.02</td>
<td>6.61</td>
<td>47.11</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>104</td>
<td>24.06 ± 1.63</td>
<td>0.05</td>
<td>14.31</td>
<td>4.65</td>
<td>0.00</td>
<td>6.00</td>
<td>48.39</td>
</tr>
</tbody>
</table>

*Refers to highly significant difference (\( P \leq 0.01 \)) between a tested and the control group.
DSBs threaten the integrity and stability of the genome. Nevertheless, they are induced endogenously during meiosis in order to provoke homologous recombination, which then repairs the breaks, generating crossover products in the process (13,15). Many studies and reviews have suggested that the generation of crossover and noncrossover products during homologous recombination is subject to control or manifests crossover homeostasis (4,15,31–34). In the present work, we induced DNA damage by whole body γ-irradiation of male mice at different sub-stages of meiotic prophase and found the irradiation increased meiotic crossovers in mouse spermatocytes.

In zygotene nuclei, synopsis is initiated between cores of homologous chromosomes and central elements of SCs begin to form. Therefore, only DNA damage introduced before pachytene can be incorporated into the meiotic recombination pathway. We evenly divided nine mice into three groups and exposed them to 1.5, 3 and 6 Gy of γ-radiation, respectively. At 24 or 48 h after irradiation, spermatocytes irradiated at late zygotene–early pachytene will have progressed to mid-pachytene, at Stages IV–VII of the cycle of the spermatogenic epithelium (16,26), and we analysed MLH1 foci in these irradiated spermatocytes compared to the control (Figure 1). Published data have revealed that MLH1 foci appear in mid-pachytene nuclei, corresponding to approximately Stage IV of the cycle of the seminiferous epithelium, and disappear at the end of pachytene, corresponding to approximately Stage VIII (20,21,35). Based on this information and the known length of the different stages of the cycle, it can be estimated that it takes at least 48 h for a cell at the zygotene/pachytene transition to develop into a mid-pachytene spermatocyte at early Stage IV (Figure 1). Therefore, the majority of cells we analysed at 24 or 48 h after exposure may have been in pachytene already at the time of irradiation, which would lead to an overestimation of the total MLH1 foci. Nevertheless, we still deduced that irradiation-induced DNA lesions at late zygotene–early pachytene were partially converted into meiotic crossovers in mid-pachytene nuclei, as could be evidenced by significant dose-dependent increases of the mean number of MLH1 foci per cell in the spermatocytes at 24 or 48 h after irradiation (Figure 2; Table I). In further analysis, these dose-dependent increases in the number of total MLH1 foci per cell were attributed to the significant dose-dependent increases in mid-pachytene, at Stages IV–VII of the cycle of the spermatogenic epithelium (16,26), and we analysed meiotic crossovers in these irradiated spermatocytes compared to the control (Figure 1). We also assumed that irradiation-induced DNA lesions at preleptotene–leptotene of prophase were at least partially incorporated into the meiotic recombination process, which led to significant dose-dependent increases in the mean number of MLH1 foci per cell in mid-pachytene spermatocyte nuclei at 120 h after irradiation (Table III). In further analysis, these dose-dependent increases in the number of total MLH1 foci per cell were attributed to the significant dose-dependent decreases in autosomal SCs with 0 MLH1 focus, and the dose-dependent increases of autosomal SCs with 2 MLH1 foci and the percentage of cells with MLH1 focus on XY bivalent (Table III).

The pairing of sex chromosomes X and Y of male placental mammals during meiosis occur only in the short pseudoautosomal regions (PARs), forming the transcriptionally silent XY bivalent during pachytene and diplotene stages of meiotic prophase (16,39). No XY bivalent was found with two or more MLH1 foci in our experiments. At 24 h after exposure, no significant dose-dependent increase was shown in the percentage of cells with MLH1 focus on XY bivalent (Table I), while at 48 or 120 h after exposure, significant dose-dependent increases were exhibited (Tables II and III), which contributed to the dose-dependent increases of the number of total MLH1 foci per cell. Therefore, the increased number of cells with an MLH1 focus on the PAR might be indicating that there is a delay in meiotic progression in the irradiated cells as the difficulty in normal meiosis for detecting these foci is often attributed to their being transient, not to their not having formed at all. Increased DNA lesions in PARs had more chances to pair with a homologous partner during synopsis and may result in dose-dependent increases in paired XY bivalents with MLH1 focus in the spermatocyte nuclei analysed. In some spermatocytes, however, if unpaired DSBs in PARs could not find a homologous partner, they may persist and result in dose-related changes in unsynapsed XY bivalents (40,41). These unpaired XY bivalents could lead to meiotic arrest (42,43).

Although significant dose-dependent increases in the number of total MLH1 foci per spermatocyte were found 24, 48 or 120 h after exposure with the gradient increase of radiation dose from 0, 1.5, 3–6 Gy, these increases were mild compared to the control groups. This would argue that there is a tight control of crossover formation (at least with respect to MLH1 foci number). Taking together all the results from this study and previous work by other researchers, we suggest that increasing doses of γ-irradiation caused dose-dependent increases of extra DSBs, which further contributed to the dose-dependent increase in MLH1 focus number in spermatocytes. Nevertheless, it is necessary to analyse such cytological markers as RAD51, DMC1, RPA and MSH4/MSH5 in the progression of meiotic recombination after irradiation in future studies.

Conclusions

In mice, the occurrence of immunofluorescent foci for mismatch repair protein MLH1 correlated closely with the occurrence of crossovers as detected genetically and MLH1 foci represent virtually all prospective crossover positions. At both 24 and 48 h after exposure, significant dose-dependent increases in the number of total MLH1 foci per spermatocyte were observed at late zygotene–early pachytene with the gradient increase of
radiation dose from 0, 1.5, 3–6 Gy. Furthermore, irradiation at preleptotene–leptotene still led to significant dose-dependent increased meiotic crossovers in the spermatocytes analysed 120 h after exposure. The increased number of cells with an MLH1 focus on the PARs may indicate that there is a delay in meiotic progression in the irradiated cells. Although significant dose-dependent increases of the number of total MLH1 foci per spermatocyte were examined 24, 48 or 120 h after exposure with the gradient increase of radiation dose from 0, 1.5, 3–6 Gy, these increases were mild compared to the control groups. This suggests that there is tight control of crossover formation (at least with respect to MLH1 foci number).

Our work raises several interesting questions. Firstly, if irradiation-induced DNA lesions lead to dose-dependent increases in meiotic crossovers in mouse mid-pachytene spermatocytes, does crossover homeostasis exist during the whole pachytene? It has been shown that DSB repair in early pachytene spermatocytes is mainly carried out through homologous recombination (HR) pathway and in late spermatocytes (late pachytene and early diplotenes) nonhomologous endjoining (NHEJ) is active (44). Secondly, if crossover homeostasis exists, when are these irradiation-induced DNA lesions repaired and are there other mechanisms for DSB repair? The mechanisms behind irradiation-induced DNA lesion repair and also cellular responses independent of DNA damage, as well as meiotic crossover homeostasis investigation in mammals, will be the subjects for future study.

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