Response of mouse thymic cells to radiation after transfusion of mesenchymal stem cells

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Abstract
Thymic lymphoma is a highly invasive and even metastatic cancer. This study investigated the effects of mesenchymal stem cells (MSCs) transfusion on cell cycle, cell proliferation, CD3 expression, mutation frequency of T cell receptor using mouse model of thymic lymphoma. C57BL/6J young mouse models of thymoma were injected with MSCs. Six months later, the thymus was taken for pathological examination and flow cytometry studies. The cells were labeled with anti-CD4, CD8, CD3, propidium iodide, or CFDA-SE, cell cycle, proliferation kinetics, and mutation frequency of T cell receptor, respectively. Pathologic results showed that control had clear corticomedullar structure with regularly shaped lymphocytes. After radiation, the thymus structure was completely destroyed, with lymphoid tumor cells diffusely distributed and heavily stained, and large nuclei. Transfusion of MSCs resulted in normal thymus structure. Cytometry studies showed that there were more CD4+/CD8- T cells in the thymus of irradiated mice than in control; transfusion of MSCs led to reduced CD4+/CD8- T cells. In irradiated mice, there were less CD4+/CD8+ T cells than in control and MSCs transfusion groups. It was observed that there were more cells arrested in G1 phase in the thymus cells and CD4+/CD8- T cells in irradiated mice than in other 2 groups, whereas there were more cells arrested in S phase in CD4+/CD8+ and CD4+/CD8- T cells in irradiated mice than in the other mice. In the thymus cells, and CD4+/CD8+ and CD4+/CD8- T cells, irradiated mice had significantly more parent, G2, G3, and G4 cells, and more cells at higher generations, and also higher proliferation index. In CD4-/CD8- T cells, irradiated mice had significantly more parent, G2, and G3 cells, and less G4, G5, G6, and propidium iodide, as compared with the other 2 groups. The expression of CD3 in CD4/CD8 T cells was significantly higher than in control. MSCs transfusion improved CD3 expression, but was still less than the control. Irradiation resulted in very high mutation frequency of T cell receptor, which was barely affected by MSCs transfusion.

Mesenchymal stem cell transfusion is able to restore the cell cycle and cell proliferation, but not CD3 expression and mutation frequency of T cell receptor in irradiated mice to control level.

Abbreviations: CD = cluster of differentiation, CFDA-SE = carboxyfluorescein diacetate succinimidyl ester, DNA = deoxyribonucleic acid, EDTA = ethylene diamine tetraacetic acid, FCM = flow cytometry, FITC = fluorescein isothiocyanate, H&E = hematoxylin and eosin, L-DMEM = low glucose Dulbecco modified eagle medium, Mf = mutation frequency, MSCs = mesenchymal stem cells, PBS = phosphate buffer saline, PE = phycoerythrin, PE-CF594 = phycoerythrin-cyanine-based fluorescent dyes594, PE-Cy5 = phycoerythrin-cyanine 5, PI = propidium iodide, RNase = ribonuclease RNAse, rpm = revolutions per minute.

Keywords: cycle cell, MSCs, mutation, proliferation, radiation, T cell receptor, thymic lymphoma

1. Introduction
Radiotherapy is 1 of the irreplaceable means for cancer therapy; however, there are increasing reports of induced secondary malignant tumors, such as thymic lymphoma, leukemia, bladder cancer, and osteosarcoma.[1–4] Thymic lymphoma induced by radiotherapy is a highly invasive and even metastatic cancer.[5]
with poor prognosis.\textsuperscript{16} Since the establishment of mouse models of radiation-induced thymoma in 1967 by Kaplan,\textsuperscript{7,17,18} many studies have been conducted regarding the induction of thymoma by radiation. For example, Mao et al\textsuperscript{9} used Hipk2 in combination with the p53 gene to treat radiation-induced thymic lymphoma; Zhao et al\textsuperscript{9} found that hydrogen protects mice from radiation-induced lymphoma. However, no effective therapy is available to treat thymic lymphoma.

Bone marrow-derived mesenchymal stem cells (MSCs) are originated in stromal cells, and can proliferate and differentiate into hematopoietic stem cells,\textsuperscript{10,11} or migrate to the injured tissue for differentiation.\textsuperscript{12} The MSCs are shown to enhance the formation of soluble factors.\textsuperscript{20,23,24} Bone marrow-derived mesenchymal stem cells (MSCs) are originated in stromal cells, and can proliferate and differentiate into hematopoietic stem cells,\textsuperscript{10,11} or migrate to the injured tissue for differentiation.\textsuperscript{12} The MSCs are shown to enhance the formation of soluble factors.\textsuperscript{20,23,24}

Since 2010, we have been using MSCs in therapy of radiation-induced thymoma, and found that MSCs reduce the incidence of thymoma.\textsuperscript{22,23} To better understand the mechanism underlying the formation of thymoma and role of MSCs in thymoma therapy, we investigated the effect of MSCs transfection on cell cycle, proliferation, CD3 expression, and mutation frequency (Mf) of T cell receptor. The findings would provide new insights for treatment of the secondary malignancies.

2. Materials and methods

2.1. MSCs culture

All protocols were approved by ethics review board of Second Hospital of Jilin University.

Neonatal C57BL/6J mice were sacrificed by cervical dislocation, and the medullary cavity was rinsed with low glucose Dulbecco modified eagle medium (L-DMEM) medium to obtain cell suspension. The cells were inoculated in 2.5 cm\textsuperscript{2} culture bottle at density of 2 × 10\textsuperscript{5} cells/mL, and cultured at 37°C and 5% CO\textsubscript{2} in incubator with saturated humidity. The half amount of medium was first refreshed after 48 hours and then after every 3 days. The cells at 90% confluence were digested with 0.25% trypsin (containing 0.02% ethylene diamine tetraacetic acid [EDTA]) for 2 minutes, and passaged at the ratio of 1:2 for 3 generations before use.

2.2. Thymoma models

Fifty-four female C57BL/6J mice (weighting 13 ± 2 g) were randomly divided into 3 groups to receive no treatment (control), radiation, and MSCs transfection. Radiation (6×MV) was given as described\textsuperscript{7} using Varian 23 EX linear accelerator (Varian Medical Systems, Inc.) at 1.75 Gy/time at dose rate of 300 Mu/min once a week for 4 weeks. On the day of last irradiation and 1 week after the irradiation, the irradiated mice were injected with 0.2 mL MSCs (2 × 10\textsuperscript{6} cells/mL) via tail veins. Six months later, the mice were sacrificed and the thymus isolated. Half of the thymus was fixed in formalin for pathological examination and the other half was grinded into single cells for flow cytometry studies.

2.3. Pathological examination

The fixed thymus was embedded in paraffin, sliced, stained with hematoxylin and eosin (H&E) and examined under microscope.

2.4. Flow cytometry

Thymus cell suspensions were filtered through 200-mesh screen and centrifuged at 1500 rpm for 5 minutes. The pellet was resuspended in 5 mL red cell lysis buffer at room temperature for 5 minutes. The lysate was centrifuged at 1500 rpm for 5 minutes and the pellet was washed twice with phosphate buffer saline (PBS). Prechilled 2 mL 70% ethanol was added to (0.2–1) × 10\textsuperscript{7} cells/mL cell suspension and fixed at 4°C overnight. The fixed cells were centrifuged at 1000 rpm for 3 minutes, washed twice with PBS, and then resuspended in 400 μL PBS, added with 50 μL of ribonuclease RNase (RNase) (1 mg/mL) and propidium iodide (PI, 400 μg/mL), and incubated at 37°C for 30 minutes. The cells were then labeled with anti-CD4-PE-CF594, anti-CD8-PITC antibodies (Becton, Dickinson and Company) at room temperature for 30 minutes in the darkness and analyzed using Beckman flow cytometry (Epics XL, Beckman Coulter Inc.) at the excitation wavelength of 488 nm according the manufacturer's instructions. The Cellquest analysis software was used to determine the DNA content and cell cycle distribution.

For analysis of cell proliferation, the cells were suspended in diluted carboxyfluorescein diacetate succinimidy ester (CFDA-SE) solution, incubated at 37°C for 10 minutes, and added with complete cell culture medium DMEM with 10% fetal bovine serum (HyClone). The cells were centrifuged at 1000 rpm for 3 minutes, washed once with complete culture medium, and incubated in complete culture medium for 5 minutes to allow unreacted CFDA-SE to release into the medium. The cells were collected by centrifugation and labeled with anti-CD4-PE-CF594 and anti-CD8-PE antibodies (Becton Dickinson). Unlabeled cells were used as control. The fluorescence intensity was measured using Beckman flow cytometer and analyzed using the Modfit software.

For analysis of CD3, 2 × 10\textsuperscript{6} to 1 × 10\textsuperscript{7} cells were incubated with anti-CD3-PE-Cy5 (Becton Dickinson), anti-CD4-PE-CF594, and anti-CD8-PE-CF594 antibodies at room temperature and darkness for 30 minutes. The cells were subjected to flow cytometry analysis, and CD3 expression was analyzed using FCS Express 4.

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\text{Mf of T cell receptor} = \frac{\text{count of CD3}^+}{\text{count of CD3}^-} \times \frac{\text{count of CD4}^+}{\text{count of CD4}^-} \times \frac{\text{count of CD8}^+}{\text{count of CD8}^-} \times \frac{\text{count of CD4}/\text{CD8}^-}{\text{count of CD4}/\text{CD8}^+}
\]

where CD3-CD4+ cells were T cell receptor mutants and CD3+/CD4+ cells were normal cells.

2.5. Statistical analysis

Each experiment was repeated at least 3 times. Data were shown as mean ± SD and analyzed using SPSS 18.0. Variance analysis was used to compare the means and differences between groups, and values with \( P < 0.05 \) or \( < 0.01 \) were considered statistically significant or highly significant.

3. Results

3.1. Differentiation thymocytes into CD4/CD8 cells

As shown in Table 1, T cells isolated were mainly CD4-/CD8-, CD4+/CD8+, or CD4+/CD8-; the proportion of CD4/CD8-subset was significantly higher in the irradiated mice than that in the control, and was reduced to normal levels in the MSCs transfection group; the proportion of CD4+/CD8+ T cells in the irradiated mice was 50.68%, significantly lower than that in the control (80.82%) and MSCs transfection group (72.85%); and
the percentage of CD4+/CD8− T cells in the transfusion group was significantly higher than that in the radiation group. There were very few CD4−/CD8+ T cells in all groups.

3.2. Effect of MSCs on the cell cycle

Cytometry analysis showed that there were similar proportions of G1 cells in MSCs transfusion and control mice (64.24% vs 69.98%; Table 2), whereas irradiated mice had significantly more G1 cells than the other 2 groups (90.53% vs 64.24% and 69.98%). There were less cells arrested in S phase in the radiation group (90.53% vs 64.24% and 69.98%), whereas irradiated mice had significantly less numbers of cells at G2 phase were similar among the 3 groups (Table 3). The numbers of cells at G2 or M phase were similar among the 3 groups. When looked at CD4+/CD8− T cells, less numbers of cells were arrested at S phase in radiation group than in control group (57.26%) and MSCs transfusion (47.35%) groups. For CD4+/CD8+ T cells, there were significantly more cells at S phase in the radiation group than in the control group, and MSCs transfusion resulted in normal numbers of these cells (Table 3). The numbers of cells at G2 or M phase were similar among the 3 groups. Furthermore, the numbers of cells at G2 phase were similar among the 3 groups (Table 3).

3.3. Effect of MSCs on the cell cycle in CD4/CD8 subsets

We further analyzed the impact of MSCs on the cell cycle in different CD4/CD8 subsets. The results showed that in CD4−/CD8+ T cells, there were significantly more G1 cells in radiation group than in control group (68.44% vs 35.73%; Table 3), and MSCs transfusion restored G1 cells to the level in control. On the contrary, significantly less numbers of cells (26.03%) were arrested at S phase in the radiation group than that in the control (57.26%) and MSCs transfusion (47.35%) groups. For CD4+/CD8+ T cells, there were significantly more cells at S phase in the radiation group than in the control group, and MSCs transfusion resulted in normal numbers of these cells (Table 3). The numbers of cells at G2 or M phase were similar among the 3 groups. When looked at CD4+/CD8− T cells, less numbers of cells were arrested at S phase in radiation group than in control group, and the number increased insignificantly after MSCs transfusion (Table 3). Furthermore, the numbers of cells at G2 phase were similar among the 3 groups (Table 3).

3.4. Effect of MSCs on the proliferation of thymocytes

Compared with control, radiation produced less cells in lower generations (<sixth generation) and more in higher generations (>sixth generation; Table 4, Fig. 1). MSCs transfusion reversed the radiation-induced effect, although not completely, resulting in cell proliferation profiles similar to the control, with similar numbers of PI-stained cells (Table 4). PI in radiation group (94.12%) was much higher than that in control group (7.27%), and MSCs transfusion greatly reduced the PI to the level (6.41%) that is even below the control (Table 4).

| Table 1 |
|---|
| Percentages of CD4/CD8 T cells in the thymus of mice irradiated with or without transfusion of MSCs. |
| Group | No. sample | CD4−/CD8− | CD4+/CD8− | CD4+/CD8+ |
|---|---|---|---|---|
| Control | 3 | 6.36±1.53 | 50.68±9.06 | 80.82±1.39 |
| Irradiation | 3 | 40.29±10.33 | 12.62±0.72 | 50.68±9.06 |
| MSCs transfusion | 3 | 9.36±6.36 | 8.94±1.39 | 72.85±10.24 |
| The symbols * and ** denote significant or highly significant difference versus control (P<0.05 or P<0.01). |
| The symbols # and ## denote significant or highly significant difference versus radiation group (P<0.05 or P<0.01). |

| Table 2 |
|---|
| Percentages of cells arrested at different cell phases after irradiation and MSCs transfusion. |
| Group | No. sample | G1 | S | G2 |
|---|---|---|---|---|
| Control | 3 | 69.98±6.40 | 22.62±3.69 | 7.40±1.38 |
| Irradiation | 6 | 90.53±3.83 | 5.77±3.17 | 3.70±1.12 |
| MSCs transfusion | 3 | 64.24±10.43 | 21.43±9.00 | 14.33±1.42 |
| The symbols * and ** denote significant or highly significant difference versus control (P<0.05 or P<0.01). |
| The symbols # and ## denote significant or highly significant difference versus radiation group (P<0.05 or P<0.01). |

| Table 3 |
|---|
| Percentages of different CD4/CD8 T cells after irradiation and MSCs transfusion. |
| CD4/CD8 subset | Group | No. sample | G1 | S | G2/M |
|---|---|---|---|---|---|
| CD4−/CD8− | Control | 3 | 35.73±6.45 | 57.26±12.34 | 6.54±11.27 |
| Irradiation | 6 | 68.44±9.83 | 26.03±4.91 | 5.53±6.37 |
| MSCs transfusion | 3 | 36.50±18.25 | 47.35±4.60 | 16.71±23.03 |
| CD4+/CD8+ | Control | 3 | 98.62±0.32 | 1.38±0.32 | 0±0 |
| Irradiation | 6 | 79.68±13.34 | 16.29±7.56 | 4.04±5.77 |
| MSCs transfusion | 3 | 88.70±4.46 | 4.34±1.86 | 6.94±3.23 |
| CD4+/CD8− | Control | 3 | 95.98±3.47 | 0.17±0.30 | 3.86±5.56 |
| Irradiation | 6 | 81.90±9.25 | 12.97±4.58 | 5.14±5.65 |
| MSCs transfusion | 3 | 88.75±6.41 | 2.27±3.95 | 8.98±6.67 |
| The symbols * and ** denote significant or highly significant difference versus control (P<0.05 or P<0.01). |
| The symbols # and ## denote significant or highly significant difference versus radiation group (P<0.05 or P<0.01). |
3.5. Effect of MSCs on the proliferation of different CD4/CD8 cells

For CD4+/CD8+ cells, analysis showed that there were more cells in the third and fourth generations, and less in the fifth and sixth generations in control and MSCs transfusion groups as compared with radiation group (Table 5, Fig. 2B), which had significantly higher PI (12.92%) than the former 2 groups (6.23% and 5.78%, respectively; Table 5).

For CD4+/CD8- cells, there were more second and third generation, and less fifth and sixth generation cells in control and MSCs transfusion groups than in radiation group (Table 5, Fig. 2C), which also had significantly higher PI (10.01%) than the former 2 groups (2.95 and 3.92%, respectively; Table 5).

### 3.6. Expression of CD3 in different CD4/CD8 cells

As shown in Table 6, 16.48% of the cells in the CD4-/CD8- subset were positive for CD3 in control, and no CD3+ T cells were detected after radiation and MSCs transfusion. The percentages of CD3+ T cells were nearly 100% in CD4+/CD8+ and CD4+/CD8- subsets in control (Table 6), and were significantly lower in radiation and MSCs transfusion groups. MSCs transfusion increased the percentages in CD4+/CD8+ and CD4+/CD8- subsets from 1.05% and 1.29% in radiation group to 7.89% and 13.23%, respectively, but was still much less than control (98.92% and 99.36%), respectively.

### 3.7. T cell receptor mutation

The profiles of CD3/CD4 expression are shown in Fig. 3, and analysis showed that mutation frequencies of the T cell receptor were very low in control (0.008%), dramatically increased in radiation group (0.996%), and MSCs transfusion slightly but not significantly increased as compared with the control group.

### Table 4

| Group | No. sample | Parent | G2 | G3 | G4 | G5 | G6 |
|-------|------------|--------|----|----|----|----|----|
| Control | 3 | 0.14 ± 0.13 | 2.28 ± 0.88 | 24.17 ± 0.92 | 47.14 ± 6.05 | 17.03 ± 6.48 | 4.76 ± 1.14 |
| Irradiation | 3 | 0.19 ± 0.21 | 0.27 ± 0.27 | 0.86 ± 0.26 | 0.85 ± 0.46 | 2.03 ± 1.30 | 3.08 ± 1.80 |
| MSCs transfusion | 3 | 1.47 ± 0.71 | 0.74 ± 0.13 | 32.01 ± 26.87 | 32.25 ± 20.65 | 0 ± 0 | 8.61 ± 4.57 |

The symbols * and ** denote significant or highly significant difference versus control (P < 0.05 or P < 0.01).

### Table 5

| Control | No. sample | Parent | G2 | G3 | G4 | G5 | G6 |
|---------|------------|--------|----|----|----|----|----|
| CD4+/CD8 - | 3 | 2.65 ± 0.97 | 13.84 ± 7.42 | 19.29 ± 18.48 | 37.54 ± 9.15 | 10.85 ± 9.40 | 11.45 ± 1.65 | 5.53 ± 0.37 |
| Irradiation | 3 | 0.03 ± 0.05 | 47.38 ± 14.71** | 40.34 ± 14.99 | 06.66 ± 0.97** | 0.99 ± 0.46 | 1.50 ± 0.35** | 2.88 ± 0.21 |
| MSCs transfusion | 3 | 2.27 ± 3.21 | 12.75 ± 5.2 | 19.09 ± 15.86 | 24.60 ± 5.19** | 28.83 ± 16.16 | 12.81 ± 3.47** | 6.24 ± 2.35** |
| CD4+/CD8 + | 3 | 0.12 ± 0.02 | 3.97 ± 0.59 | 28.32 ± 2.69 | 46.90 ± 1.25 | 14.10 ± 1.45 | 4.45 ± 0.89 | 6.23 ± 0.27 |
| Irradiation | 3 | 0.32 ± 0.34 | 0.66 ± 0.70 | 4.66 ± 5.15** | 19.77 ± 7.98** | 37.77 ± 0.72** | 36.82 ± 2.91** | 12.92 ± 1.36** |
| MSCs transfusion | 3 | 0.16 ± 0.04 | 1.58 ± 2.58 | 38.32 ± 11.24** | 54.22 ± 10.79** | 4.27 ± 3.25** | 0.65 ± 0.09** | 5.78 ± 0.78** |

The symbols * and ** denote significant or highly significant difference versus control (P < 0.05 or P < 0.01).

The symbols # and ## denote significant or highly significant difference versus radiation group (P < 0.05 or P < 0.01).
insignificantly reduced the frequency as compared with the radiation group (Table 7).

### 4. Discussion

It has been shown that mouse thymus T cells are derived from the bone marrow,\[^{27}\] they migrate in different parts of the thymus gland to differentiate and mature, and are released into the blood for immune response. Most studies suggest that immature thymus T cells normally develop into T cell lymphoma continuously, when CD4-/CD8- T cells are differentiated into CD4+/CD8- T cells and CD4-/CD8+ T cells from CD4+/CD8+ T cells.\[^{28}\] Some differences in the percentages of CD4/CD8 subsets have been noticed in different mouse strains and at different differentiation and maturation stages; however, the maturation process is consistent. Our study showed a significant increase in CD4-/CD8- T cells after radiation. This is different from results in an earlier work, where CD4-/CD8- cells were reduced and CD4+/CD8+ or CD4-/CD8+ cells were increased after adult mice were irradiated with x-rays.\[^{29}\] The difference may result from the differences in radiation dose applied, mouse genotype used, and detection times postirradiation. In our study, we analyzed the samples 6 months after irradiation, whereas in the early study, analysis was conducted 32 days after irradiation.\[^{29}\]

#### Table 6

| Group          | n | CD4-/CD8- (%) | CD4+/CD8+ (%) | CD4+/CD8- (%) |
|----------------|---|---------------|---------------|---------------|
| Control        | 3  | 16.48 ± 3.18  | 98.92 ± 0.20  | 99.36 ± 0.07  |
| Irradiation    | 6  | 0 ± 0 **      | 1.05 ± 0.37 **| 1.29 ± 1.08 **|
| MSCs transfusion| 3  | 0 ± 0 **      | 7.89 ± 8.59 **| 13.23 ± 6.63 **|

The symbols * and ** denote significant or highly significant difference versus control (P < 0.05 or P < 0.01). The symbols # and ## denote significant or highly significant difference versus radiation group (P < 0.05 or P < 0.01).
showed that there are some differences in the percentages of CD4/CD8 subsets in different mouse strains and at different differentiation and maturation stage, although the maturation process is consistent.\[^{30}\] To elucidate the inhibitory effect of MSCs on thymoma, we analyzed the effect of MSCs on cell cycle and proliferation. It is found that in the irradiated mice, the cell arrest was mainly at G1 phase. This is consistent with early results that x-ray irradiation results in arrest of thymic cells at G1 phase in mice.\[^{31}\] This may be due to the irradiation-induced DNA damage or repair error in the thymus tissue, which triggers cells to respond by activating the cell cycle arrest mechanism that arrests the cells at G1 phase to initiate DNA repair mechanism to repair DNA damage. Similarly, CD4+/CD8- T cells were also mainly arrested at G1 phase after radiation. This is probably because the immature CD4-/CD8- T cells in the thymus are very sensitive and vulnerable to radiation. Arrest at G1 phase would prevent the errors from being transmitted to next generation. On contrast, for the relatively mature CD4+/CD8+ and CD4+/CD8- T cells, radiation mainly increased the cells arrested at S phase, suggesting that radiation simulates the advance and proliferation of cells as compared with control and MSCs transfusion. This also implies that high proliferation of tumor is mainly due to the increased proliferation of mature cells.

The proliferation kinetics analysis showed that in the irradiated mice, thymocytes became more proliferative, which is particularly remarkable for CD4+/CD8- and CD4+/CD8- subsets. Early study showed that 24 hours after x-ray irradiation, there were less CD4+/CD8- and more CD4+/CD8+ and CD4+/CD8- cells in mice.\[^{32}\] MSCs transfusion reduced the proliferation to near control level. On the contrary, proliferation of the CD4-/CD8- T cells in the irradiated mice were less than those in control and MSCs transfusion groups, suggesting that the CD4-/CD8- T cells in the irradiated mice are immature cells released from the bone marrow after irradiation and they are less proliferative.

We also analyzed the percentage of CD3+ cells in different subsets of CD4/CD8 cells as a measure of cell maturity. The results showed that 16.48% of cells in the CD4-/CD8-subset were mature in control, but none of them was found in the irradiated or MSCs-transfused mice; for CD4+/CD8+ and CD4+/CD8- subsets, the mature cells were close to 100%, whereas they were only about 1% after irradiation. Su et al\[^{32}\] found that the number of CD3+ thymocytes is highly correlated to the total number of CD4+ and CD8+ cells. MSCs transfusion increased the percentages of CD3+ cells, but the percentages were still much lower than those in control, suggesting that the cell population after fractionated irradiation is mainly composed of immature cells, and is mainly mature cells after single low-dose irradiation, as a result of cell supplement from bone marrow,\[^{33}\] and such situation is not able to be restored completely by the MSCs transfusion used in the study. Our work demonstrated that radiation increases the Mf of T-cell receptor.\[^{34}\] As expected, dramatic increase in the mutation frequency was observed after irradiation in our experiments. These radiation-induced mutations were barely affected by MSCs transfusion, suggesting they are irreversible.

Taken together, our results show that MSCs transfusion to irradiated mice restores the cell cycle and proliferation kinetics to normal levels; however, it has limited impact of CD3 expression and no effect on the mutation frequency of T cell receptor. These findings suggest that MSCs may adjust the cellular microenvironment to restore normal cell growth after radiation and demonstrate the cellular roles of MSCs in the prevention and treatment of thymoma.

**Table 7**

| Group            | No. sample | Mutation frequency (%)     |
|------------------|------------|---------------------------|
| Control          | 3          | 0.008±0.001               |
| Irradiation      | 6          | 0.996±0.003**             |
| MSCs transfusion | 3          | 0.989±0.001**             |

* Denote significant or highly significant difference versus control (P<0.01).

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