Hematopoietic Stem Cells from Ts65Dn Mice Are Deficient in the Repair of DNA Double-Strand Breaks

Yingying Wang,^{a,b} Jianhui Chang,^b Lijian Shao,^b Wei Feng,^b Yi Luo,^b Marie Chow,^c Wei Du,^b Aimin Meng^a and Daohong Zhou^{b,1}

^a Tianjin Key Laboratory of Radiation Medicine and Molecular Nuclear Medicine, Institute of Radiation Medicine, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin 300192, China; and ^b Division of Radiation Health, Department of Pharmaceutical Sciences and ^c Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205

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Down syndrome (DS) is a genetic disorder caused by the presence of an extra partial or whole copy of chromosome 21. In addition to musculoskeletal and neurodevelopmental abnormalities, children with DS exhibit various hematologic disorders and have an increased risk of developing acute lymphoblastic leukemia and acute megakaryocytic leukemia. Using the Ts65Dn mouse model, we investigated bone marrow defects caused by trisomy for 132 orthologs of the genes on human chromosome 21. The results showed that, although the total bone marrow cellularity as well as the frequency of hematopoietic progenitor cells (HPCs) was comparable between Ts65Dn mice and their age-matched euploid wild-type (WT) control littermates, human chromosome 21 trisomy led to a significant reduction in hematopoietic stem cell (HSC) numbers and clonogenic function in Ts65Dn mice. We also found that spontaneous DNA double-strand breaks (DSBs) were significantly increased in HSCs from the Ts65Dn mice, which was correlated with the significant reduction in HSC clonogenic activity compared to those from WT controls. Moreover, analysis of the repair kinetics of radiation-induced DSBs revealed that HSCs from Ts65Dn mice were less proficient in DSB repair than the cells from WT controls. This deficiency was associated with a higher sensitivity of Ts65Dn HSCs to radiation-induced suppression of HSC clonogenic activity than that of euploid HSCs. These findings suggest that an additional copy of genes on human chromosome 21 may selectively impair the ability of HSCs to repair DSBs, which may contribute to DS-associated hematological abnormalities and malignancies. © 2016 by

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INTRODUCTION

Down syndrome (DS) is one of the most common chromosomal abnormalities in humans, caused by the presence of an extra partial or entire copy of chromosome 21, and occurs in about one of every 700 babies born each year (1). DS is caused by genomic-dosage imbalance, which may directly or indirectly alter the timing, pattern or extent of the disease (2, 3). In addition to musculoskeletal and neurodevelopmental abnormalities and other health problems, children with DS are also at a higher risk of developing acute leukemia, poor immune function and premature aging. It has been proposed that defects in hematopoietic stem cells (HSCs) and lymphoid progenitor cells may account for the immune dysfunction in DS patients (4). However, the mechanism underlying the higher risk of acute leukemia in DS remains to be elucidated.

Aneuploidy can impair DNA damage repair and lead to genomic instability (5–7). Genetic instability resulting from defects in DNA damage repair, particularly in the repair of DNA double-strand breaks (DSBs), and can contribute to tumorigenesis (8). However, whether the trisomy of human chromosome 21 affects the repair of DNA damage is still controversial. For example, it has been shown that peripheral blood lymphocytes from DS patients are defective in the repair of DNA-damaging agent-induced DNA single-strand breaks (SSBs) and base excision repair (BER) (9) [reviewed in ref. (10)]. Previously published studies have also shown that lymphocytes from DS patients were less efficient in the repair of radiation-induced SSBs (11). In contrast, the published work by Leonard and Mertz (12), and Steiner and Woods (13) demonstrated that lymphocytes and fibroblasts from DS patients and non-DS individuals had a similar ability to repair SSBs and DSBs.

The Ts65Dn mouse is trisomic for 132 orthologs of the genes on human chromosome 21 (14–16) and is one of the most widely used mouse models for DS research. This mouse model recapitulates the various deficiencies similar to those seen in DS patients, including learning impairments and behavioral deficits, as well as immunodeficiency,

¹ Address for correspondence: University of Arkansas for Medical Sciences, Department of Pharmaceutical Sciences, 4301 W. Markham, no. 607, Little Rock, AR 72205; email: DZhou@uams.edu.

mental retardation and increased cancer incidence (15, 17–20). Previously published studies have demonstrated a significant reduction in HSC numbers and function along with an increased HSC production of reactive oxygen species (ROS) in Ts65Dn mice compared to euploid, wild-type (WT) control littermates (4). In the current study, we investigated DSB repair in HSCs and hematopoietic progenitor cells (HPCs) from Ts65Dn and wild-type mice. Our results showed HSCs, but not HPCs, from Ts65Dn mice exhibited a significant increase in spontaneous DSBS and delayed repair of radiation-induced DSBS in HSCs. These changes in Ts65Dn mice were associated with a significant reduction in HSC clonogenic function and a higher sensitivity of HSCs to radiation-induced suppression compared with the cells from wild-type mice. Therefore, our study has identified a novel link between trisomy chromosome 21 and HSC dysfunction via DSB repair.

MATERIALS AND METHODS

Mice

Ts65Dn mice and their age-matched euploid, WT control littermates (8–10 weeks old) were purchased from Jackson Laboratories (Bar Harbor, ME). Mice were housed at the University of Arkansas for Medical Sciences (UAMS) AAALAC-certified animal facility (Little Rock, AK), and received food and water *ad libitum*. The Institutional Animal Care and Use Committees of UAMS approved all experimental procedures used in this study.

Antibodies

The following were purchased from BD Biosciences (San Jose, CA): Purified rat anti-mouse Ter119 (cat. no. 553671), CD11b (cat. no. 553308), Gr1 (cat. no. 553123), CD3e (cat. no. 553238) and B220 antibodies (cat. no. 553084); biotin-conjugated rat anti-mouse Ter119 (cat. no. 553672), CD11b (cat. no. 557395), Gr1 (cat. no. 553125), CD3e (cat. no. 553239) and B220 antibodies (cat. no. 553086); FITC-conjugated streptavidin (cat. no. 554060); PE-conjugated anti-Sca1 antibody (cat. no. 553108); and APC-H7-conjugated anti-c-Kit antibody (cat. no. 560185). The following were purchased from BioLegend® Inc. (San Diego, CA): Pacific blue-conjugated anti-CD48 antibody (cat. no. 103418) and APC-conjugated anti-CD150 antibody (cat. no. 115910). Alexa Fluor® 488-conjugated rabbit anti-phosphohistone H2A.X (Ser139) (20E3) mAb (cat. no. 9719) was purchased from Cell Signaling Technology® (Danvers, MA). Anti-phosphohistone H2A.X (Ser139) antibody (cat. no. 05-636) was purchased from EMD Millipore (San Diego, CA). Alexa Fluor 488-conjugated goat anti-mouse IgG (H+L) secondary antibody (cat. no. A-11001) was purchased from Thermo Fisher Scientific™ Inc. (Carlsbad, CA).

Isolation of Bone Marrow Mononuclear Cells and Lineage-Negative Hematopoietic Cells

The femora and tibiae were harvested from the mice immediately after they were euthanized by CO₂. Bone marrow cells (BMCs) were flushed from the bones into Hank's balanced salt solution (HBSS, cat. no. 14170-112; Thermo Fisher Scientific) containing 2% fetal bovine serum (FBS, cat. no. S11050H; Atlanta Biologicals, Flowery Branch, GA) using a 23-gauge needle and syringe (cat. no. 309571; BD Biosciences). BMCs were centrifuged through Histopaque®-1083 (Sigma-Aldrich® LLC, St. Louis, MO) to isolate bone marrow mononuclear cells (BM-MNCs). To isolate lineage-negative hematopoietic cells (Lin⁻ cells), BM-MNCs were incubated with rat

antibodies specific for murine CD3e, CD11b, B220, Ter-119 and Gr-1. The labeled mature lymphoid and myeloid cells were depleted by incubation with goat anti-rat IgG paramagnetic beads (Dynal Inc., Lake Success, NY) at a bead to cell ratio of approximately 4:1. Cells binding the paramagnetic beads were removed with a magnetic field. The negatively isolated Lin⁻ cells were washed twice with 2% FBS/HBSS and resuspended in 2% FBS/HBSS.

Analysis of Frequencies and Numbers of HPCs and HSCs by Flow Cytometry

Analysis of frequencies and numbers of HPCs and HSCs were performed as previously described elsewhere (21). Briefly, BM-MNCs were pre-incubated with biotin-conjugated anti-Ter119, anti-CD11b, anti-Gr1, anti-CD3e and anti-B220 antibodies. They were then stained with streptavidin-FITC and Sca1-PE, c-Kit-APC-H7, CD150-APC and CD48-Pacific Blue antibodies. The frequencies of HPCs (Lin⁻ Sca1⁻ c-Kit⁺ cells), Lin⁻ Sca1⁺ c-Kit⁺ cells (LSK cells), and HSCs (CD150⁺ CD48⁻ LSK cells) were analyzed by flow cytometry with an Aria II™ cell sorter (BD Biosciences) and FlowJo software (Ashland, OR).

Analysis of Gamma-H2AX Staining in HPCs and HSCs by Flow Cytometry

Lin⁻ cells (1 × 10⁶ per sample) were resuspended with RPMI 1640 media (ATCC®, Manassas, VA) supplemented with 10% FBS, 100 µg/ml streptomycin and 100 U/ml penicillin (P/S; Atlanta Biologicals). They were sham or 2 Gy irradiated in a JL Shepherd Mark I ¹³⁷cesium gamma-irradiator (Glendale, CA) at a dose rate of 0.916 Gy/min on a rotating platform. The cells were cultured at 37°C with 5% CO₂ and 100% humidity for various durations before they were harvested for immunostaining. Specifically, the cells harvested from culture were stained with antibodies against various HSC surface markers, fixed and then permeabilized using Fixation/Permeabilization solution (BD Pharmingen™, San Diego, CA). Subsequently, they were stained with Alexa Fluor 488-conjugated rabbit anti-γ-H2AX (Ser139) antibody (Cell Signaling Technology) and analyzed by flow cytometry.

Gamma-H2AX Foci Assay

Approximately 2,000 freshly sorted sham- or 2 Gy irradiated HPCs or HSCs were spun on a slide for immunostaining by Cytospin™. After being fixed with 4% paraformaldehyde for 15 min, cells were incubated in 0.1% Triton™ X-100 at room temperature for 30 min. After being washed with PBS and a 60 min incubation in PBS/1.5% bovine serum albumin, cells were incubated overnight at 4°C with anti-γ-H2AX (1:1000) antibody and then with corresponding secondary antibodies with extensive washing between each step. The nuclear DNA of the cells was counterstained with DAPI (cat. no. D9542; Sigma-Aldrich). The cells were viewed and photographed using an Axioplan research microscope (Carl Zeiss Inc., Jena, Germany) equipped with a 100 W mercury light source. The images were captured with a CCD100 integrating camera (Dage-MTI Inc., Michigan City, IN) and a Flashpoint® 128 capture board (Integral® Technologies, Indianapolis, IN). The captured images were processed using Image-Pro® Plus software (Media Cybernetics Inc., Rockville, MD) and displayed with Adobe Photoshop v.6.0. A total of more than 100 cells per slide were counted in >30 random fields on a slide to determine the number of γ-H2AX foci for calculation of average number of γ-H2AX foci/cell.

Single HPC and HSC Colony-Forming Cell Assay

Single HPCs and HSCs from Ts65Dn and wild-type mice were directly sorted into wells of round-bottom 96-well plates at one cell/well. HPCs were cultured in RPMI 1640 media supplemented with

FIG. 1. Ts65Dn mice exhibit significant decreases in HSC frequency and clonogenic function compared to wild-type mice. Panel A: Represents flow cytometric analyses of HPCs (Lin⁻ Sca1⁻ c-Kit⁺ cells), LSK cells (Lin⁻ Sca1⁺ c-Kit⁺ cells) and HSCs (CD150⁺ CD48⁻ LSK cells) in BM-MNCs from Ts65Dn mice or their aged matched euploid control littermates [wild-type (WT)]. Panel B: Number of BM-MNCs and Lin⁻ cells in Ts65Dn and wild-type mice. Panel C: Frequency of HPCs, LSK cells and HSCs in Lin⁻ cells from Ts65Dn and wild-type mice. Panel D: Number of HPCs, LSK cells and HSCs in Ts65Dn and wild-type mice. Panel E: Percentage of colony-forming HPCs in single-sorted HPCs from Ts65Dn and wild-type mice. Panel F: Percentage of colony-forming HSCs in single-sorted HSCs from Ts65Dn and wild-type mice. The data are presented as mean ± SD (n = 3 mice/group). **P* < 0.05 and ***P* < 0.01 vs. WT.

20% FCS, 25 ng/ml of SCF, Flt3, IL-11 and TPO, 10 ng/ml GM-CSF and IL-3, 4 U/ml EPO (PeproTech®, Rocky Hill, NJ), 0.1 mM nonessential amino acids (cat. no. 11140-050), 1 mM sodium pyruvate (cat. no. 11360-070), 2 mM L-glutamine (cat. no. 25030-081; Thermo Fisher Scientific) and 50 μM 2-mercaptoethanol (cat. no. M6250; Sigma-Aldrich). HSCs were cultured in RPMI 1640 media supplemented with 10% FCS, 50 ng/ml of SCF, Flt-3, TPO and GM-CSF, 20 ng/ml of IL-3 and 5 U/ml EPO (PeproTech). Freshly prepared media was added every 3 days. After 14 days of culture, the numbers of cells produced by each HPC or HSC were counted. Single HPCs and HSCs producing more than 100 cells and 10,000 cells were scored as colony-forming HPCs and HSCs, respectively.

Statistical Analysis

The data were analyzed by analysis of variance (ANOVA). For experiments in which only single experimental and control groups were used, group differences were examined by unpaired Student *t* test. Differences were considered significant at *P* < 0.05. All analyses were performed using GraphPad Prism (GraphPad Software Inc., San Diego, CA).

RESULTS

Ts65Dn Mice Exhibit Significant Decreases in HSC Frequency and Clonogenic Function Compared to WT Mice

To characterize the hematopoietic abnormalities of Ts65Dn mice, we performed phenotypic analysis of various populations of hematopoietic stem and progenitor cells in BMCs from Ts65Dn mice and wild-type mice by flow cytometry (Fig. 1A). The results showed comparable bone marrow cellularity among Ts65Dn mice and their age-matched WT controls (Fig. 1B). Interestingly, although no significant difference was found in Lin⁻ cells and HPC compartment of Ts65Dn mouse bone marrow, Ts65Dn mice exhibited a significant reduction in both the percentage and absolute number HSCs compared to WT controls (Fig. 1A–D). These results are in line with previous studies (4, 16, 22). We then assessed the clonogenic function of HPCs and HSCs from Ts65Dn

FIG. 2. HPCs and HSCs from Ts65Dn mice exhibit significant increases in the basal levels of DSBs compared to HPCs and HSCs from wild-type mice. Panel A: Analysis of DSBs in HPCs and HSCs by γ -H2AX immunostaining and flow cytometry. Left side: Representative flow cytometric analyses of γ -H2AX immunostaining. Right side: MFI of γ -H2AX immunostaining. ISO = isotype control antibody. Panel B: γ -H2AX foci assay. Left side: Represents immunofluorescence images of γ -H2AX foci in HPCs and HSCs. Right side: The average number of γ -H2AX foci per cell. Panels C and D: The distribution of HPCs and HSCs with different numbers of γ -H2AX foci per cell. Panel E: Percentage of HPCs and HSCs with more than two γ -H2AX foci per cell. The data are presented as mean $\pm$ SD (panel A, $n = 6$ mice/group; and panels B–E, 3 independent assays with pooled HSCs and HPCs from 3 mice for each assay). * $P < 0.05$ and ** $P < 0.01$ vs. WT.

and wild-type mice by a single-cell clonogenic assay (23). We found that about 47% of single HPCs sorted from Ts65Dn mice could form a colony after 14 days of culture, which was comparable with those from the wild-type mice (51%) (Fig. 1E). However, the colony-forming ability of HSCs from Ts65Dn mice was significantly reduced compared to that from wild-type mice, e.g., from 72% in WT HSCs to 49% in Ts65Dn HSCs (Fig. 1F). These results confirm that Ts65D mice have a HSC-specific defect.

Ts65Dn Mouse HPCs and HSCs Exhibit Significant Increases in DSB Basal Levels Compared to Wild-Type Mouse HPCs and HSCs

In their published work, Sheltzer *et al.* reported that aneuploid fission yeasts were found to be deficient in DNA

damage repair (5). Defects in DNA damage repair, particularly DSB repair, can lead to HSC dysfunction [reviewed in ref. (24)]. To determine whether the decline in HSC pool and clonogenic function in Ts65Dn mice is associated with a defect in HSC DSB repair, we first measured the basal levels of DSBs in HPCs and HSCs from Ts65Dn and wild-type mice. This was done by flow cytometric analysis of γ -H2AX immunostaining in HPCs and HSCs, since γ -H2AX is the most commonly used biomarker for DSBs (25). As shown in Fig. 2A, HPCs and HSCs from Ts65Dn mice exhibited about 20 and 37% higher γ -H2AX mean fluorescent intensity (MFI) than the cells from wild-type mice, respectively. These results suggest that both HPCs and HSCs from Ts65Dn mice have significantly higher basal levels of DSBs than the cells from wild-type mice. To confirm this finding, we next

performed γ -H2AX foci assay using sorted HPCs and HSCs from Ts65Dn and wild-type mice. The results from this assay showed that the number of γ -H2AX foci in HSCs from Ts65Dn was significantly increased compared to that in HSCs from wild-type mice (Fig. 2B). The number of γ -H2AX foci in HPCs from Ts65Dn was also slightly increased compared to that in HPCs from wild-type mice, but was not statistically significant. In addition, we also quantified the percentage of HPCs and HSCs with different numbers of γ -H2AX foci and found that the percentages of HPCs and HSCs containing more than two γ -H2AX foci were increased from 24% and 9% in wild-type mice, respectively, to 31% and 18% in Ts65Dn mice, respectively (Fig. 2C–E). Taken together, our data demonstrate that HPCs and HSCs from Ts65Dn mice have an elevated DSB basal level compared to those from wild-type mice. The increase in the DSB basal levels in Ts65Dn HSCs (50%) was much greater than in Ts65Dn HPCs (29%).

Ts65Dn Mouse HSCs Are Less Proficient in Repairing Radiation-Induced DSBs than Wild-Type Mouse HSCs

To determine whether the increases in the basal levels of DSBs in HPCs and HSCs from Ts65Dn mice result from a defect in DSB repair, we isolated Lin[−] cells from Ts65Dn and wild-type mice and then exposed the cells to 2 Gy. The repair of radiation-induced DSBs in HPCs and HSCs was analyzed by γ -H2AX immunostaining and flow cytometry at different time points after the exposure. The results showed that there were no significant differences in the peak levels of γ -H2AX staining in HPCs and HSCs from both Ts65Dn and wild-type mice, indicating that HPCs and HSCs from Ts65Dn and wild-type mice are equally sensitive to radiation-induced DSBs (Fig. 3A). The MFI of γ -H2AX staining in HPCs from Ts65Dn and wild-type mice declined rapidly in a similar manner after irradiation, indicating an efficient repair of DSBs by HPCs from Ts65Dn and wild-type mice. However, HSCs from Ts65Dn mice showed a significant delay in the reduction of γ -H2AX staining after irradiation compared to HSCs from wild-type mice, suggesting that Ts65Dn HSCs are less proficient in the repair of radiation-induced DSBs than WT HSCs. This was confirmed by γ -H2AX foci assay. The results from this assay showed that radiation-induced DSBs in HPCs and HSCs peaked approximately 1 h postirradiation and the majority of the DSBs in HPCs and HSCs from wild-type mice were repaired within 6 h postirradiation (Fig. 3B and C). Although Ts65Dn mouse HPCs were equally proficient in the repair of radiation-induced DSBs as wild-type mouse HPCs, Ts65Dn mouse HSCs exhibited a significant delay in the repair, confirming that HSCs from Ts65Dn mice are deficient in the repair of DSBs compared to HSCs from wild-type mice (Fig. 3B and C).

Ts65Dn Mouse HSCs Are More Sensitive to Radiation-Induced Suppression of Clonogenic Function than Wild-Type Mouse HSCs

To determine whether DSB repair deficiency increased radiosensitivity in HSCs from the Ts65Dn mouse, we analyzed the clonogenic function of HSCs and HPCs from Ts65Dn and wild-type mice after 2 Gy exposure, using the single-cell clonogenic assay. As shown in Fig. 4A, approximately 50% of nonirradiated HPCs from Ts65Dn and wild-type mice were capable of forming a colony. The colony-forming HPCs reduced to about 25% after irradiation regardless of whether they were from Ts65Dn or wild-type mice, indicating that HPCs from Ts65Dn or wild-type mice are equally sensitive to radiation. As shown in Fig. 4B, significantly fewer HSCs from Ts65Dn mice had the ability to form a colony than HSCs from wild-type mice without exposure to radiation. The colony-forming ability was dramatically reduced in HSCs from Ts65Dn and wild-type mice after 2 Gy exposure. However, the reduction was significantly greater in Ts65Dn mouse HSCs than in wild-type mouse HSCs. This result indicates that HSCs from Ts65Dn mice are more sensitive to radiation than HSCs from wild-type mice, possibly due in part to a DSB repair defect in Ts65Dn mouse HSCs.

DISCUSSION

While Down syndrome has been recognized as one of the most important leukemia-predisposing syndromes (26–32), the mechanisms underlying this predisposition remain unclear. Several DS mouse models have been developed to identify dosage-sensitive genes that contribute to the DS-related hematopoietic phenotypes. Among them, the Ts65Dn mouse, trisomic for 132 orthologous genes of human chromosome 21, represents one of the best models for studying hematological alterations caused by trisomy of human chromosome 21 in mice, because it develops macrocytic anemia and myeloproliferative disorder associated with thrombocytosis similar to that seen in DS patients (22). A previously published study using this mouse model demonstrated an accelerated stem cell aging phenotype, which leads to HSC and lymphoid progenitor cell defects and immune dysfunction (4). In line with previous studies (4, 16, 22), we investigated hematological alterations in Ts65Dn mice and found a significantly reduced number and impaired clonogenic function of HSCs but not HPCs in Ts65Dn mouse bone marrow. These findings support the notion that trisomy of human chromosome 21 affects HSC function in mice.

More important, using Ts65Dn mice we investigated whether trisomy of human chromosome 21 in mice can alter the ability of HSCs to repair DSBs. This is because aneuploidy can impair DNA damage repair to cause genomic instability (5–7), and defects in DNA damage repair, particularly DSB repair, can cause HSC dysfunction.

FIG. 3. HSCs from Ts65Dn mice are less proficient in the repair of radiation-induced DSBs than HSCs from wild-type mice. Panel A: DSB repair kinetics in HPCs and HSCs were analyzed by γ -H2AX immunostaining and flow cytometry after 2 Gy irradiation. Data at different time points were normalized to the MFI of γ -H2AX immunostaining at 1 h postirradiation. Panel B: γ -H2AX foci assay. Representative of immunofluorescence images from γ -H2AX foci assay in HPCs and HSCs after sham irradiation (control) or 1 and 6 h postirradiation (2 Gy). Panel C: Average number of γ -H2AX foci per cell. The data are presented as mean $\pm$ SD (panel A, $n = 3$ mice/group; and panels B and C, 3 independent assays with pooled HSCs and HPCs from 3 mice for each assay). * $P < 0.05$ vs. WT.

The results from our study showed that HSCs from Ts65Dn mice exhibited a significantly higher level of DSBs at a homeostatic condition than HSCs from wild-type mice. The increase in DSBs may be, in part, attributable to an increased production of ROS by Ts65Dn HSCs [(4) and data not shown]. In addition, a defect in the repair of DSBs can also contribute to the increase in DSBs in Ts65Dn mouse HSCs. Indeed, the results from our study showed that HSCs from Ts65Dn mice were less proficient in repair

of radiation-induced DSBs than HSCs from wild-type mice. Moreover, the deficiency in DSB repair was associated with a higher sensitivity of Ts65Dn HSCs to radiation-induced suppression of clonogenic function. These findings provide new insights into the mechanisms by which trisomy of human chromosome 21 affects HSC function.

However, which of the trisomic 132 orthologous genes of human chromosome 21 in Ts65Dn mouse model is responsible for impairing the ability of HSCs to repair

FIG. 4. HSCs from Ts65Dn mice are more sensitive to radiation-induced suppression of clonogenic function than HSCs from wild-type mice. Panel A: Percentage of colony-forming HPCs in single-sorted HPCs from Ts65Dn and wild-type mice after sham (control) or 2 Gy irradiation *in vitro*. Panel B: Percentage of colony-forming HSCs in single-sorted HSCs from Ts65Dn and wild-type mice after sham (control) or 2 Gy irradiation *in vitro*. The data are presented as mean $\pm$ SD (n = 3 mice per group). * P < 0.05 and *** P < 0.001 vs. WT.

DSBs remains unidentified. A recent study using Ts65Dn and Ts1Cje DS mouse models revealed that triplication of *Usp16* may contribute to HSC dysfunction in Ts65Dn mice, in part by decreasing ubiquitination of Cdkn2a and accelerating senescence (16). In addition, USP16 is an important deubiquitinase that has been implicated in regulation of DNA damage response [reviewed in (33)] through downregulation of the RNF8-RNF168 pathway [(34); reviewed in refs. (33, 35)]. It would be very valuable to determine whether USP16 may also play a role in the impairment of DSB repair in Ts65Dn HSCs.

The deficiency in DSB repair in HSCs may contribute to the predisposition of DS patients to certain hematological disorders and malignancies, because an inability to efficiently repair DSBs can lead to genetic instability and HSC dysfunction. HSC dysfunction can compromise the fitness of HSCs. The decrease in HSC fitness may in turn promote clonal hematopoiesis to allow HSCs with oncogenic mutations and genetic instability to expand and accumulate more mutations for transformation (36). There-

fore, a better understanding of the underlying molecular mechanisms by which trisomy of human chromosome 21 impairs DSB repair in HSCs may lead to new strategies to prevent DS-associated hematological abnormalities.

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