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Telomere dysfunctional environment induces loss of quiescence and inherent impairments of hematopoietic stem cell function

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Summary

Previous studies have shown that telomere dysfunction induces alteration in the systemic (circulatory) environment impairing the differentiation of hematopoietic stem cells (HSCs) but these defects can be reverted by re-exposing HSCs to an environment with functional telomeres. In contrast, HSC intrinsic telomere dysfunction induces permanent and irreversible limitations in the repopulation capacity partially depending on the induction of checkpoints such as cell cycle arrest, differentiation, or apoptosis. It is currently unknown whether telomere dysfunctional environment can induce irreversible, cell intrinsic defects impairing the function of HSCs. Here, we analyzed the functional reserves of murine, wild-type HSCs with intact telomeres that were transiently exposed to a telomere dysfunctional environment (late generation telomerase knockout mice) or to an environment with functional telomeres (wild-type mice). The study shows that the telomere dysfunctional environment leads to irreversible impairments in the repopulation capacity of wild-type HSCs. The telomere dysfunctional environment impaired the maintenance of HSC quiescent. Moreover, the study shows that alterations in the systemic (circulatory) environment rather than the bone stromal niche induce loss of stem cell quiescence and irreversible deficiencies of HSCs exposed to a telomere dysfunctional environment.

Key words: telomere; aging; stem cell; quiescence; systemic environment.

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Introduction

Stem cells contribute to tissue maintenance over the lifetime of an individual. Experimental data indicate that cell intrinsic and cell extrinsic factors can contribute to the decline in stem cell function during aging: (i) It was shown that hematopoietic stem cells (HSCs) from aged donor mice exhibit a significantly reduced function compared with HSCs from young donor mice in transplantation assays (Morrison *et al.*, 1996; Sudo *et al.*, 2000). These deficiencies were associated with age-associated changes in gene expression profiles of HSCs from aged vs. young mice (Rossi *et al.*, 2005; Chambers *et al.*, 2007) and have led to the concept that HSC aging can be induced by cell intrinsic factors. (ii) Experimental evidence from different model systems indicated that both the decline in niche function and alterations in the systemic environment could contribute to the impairment of stem cell maintenance and function during aging (Conboy *et al.*, 2005; Boyle *et al.*, 2007; Pan *et al.*, 2007). Specifically, it was shown that aged stem cells rejuvenate by exposure to a young systemic environment in parabiosis experiments (Conboy *et al.*, 2005). *Vice versa*, exposure of young stem cells to an aged environment led to stem cell aging. Together, these evidences indicate that both cell intrinsic and cell extrinsic processes can contribute to the decline in stem cell function during aging. It is conceivable that there are cross-connections between intrinsic and extrinsic mechanisms of stem cell aging. Along these lines, it is also possible that alterations in the cellular environment could lead to intrinsic alterations in stem cells that may also persist even if the environment would be ''rejuvenated''. However, such cross-talks have so far not been demonstrated. To address this question, it appears to be important to use defined models of aging induced by specific molecular processes, such as DNA damage.

An accumulation of DNA damage was observed in HSCs during mouse and human aging, (Rossi *et al.*, 2007; Rube *et al.*, 2011). Genetically engineered mouse models revealed experimental evidence that the accumulation of DNA damage and telomere dysfunction can contribute to the age-related decline of stem cell maintenance and function (Choudhury *et al.*, 2007; Rossi *et al.*, 2007). Telomeres are specialized complexes located at chromosomal ends. The main function of telomeres is to protect the chromosomal ends and prevent DNA damage responses (Blackburn, 2001; de Lange, 2002). Telomere shortening occurs with human aging and limits the proliferation capacity of primary human cells to a finite number of cell divisions (Allsopp *et al.*, 1992). Critically, short telomeres lose the capping function at the chromosomal ends and these dysfunctional telomeres induce DNA damage checkpoints leading to cell cycle arrest or apoptosis. Organ systems with high rates of cell turnover are most sensitive to telomere shortening in mice and humans (Lee *et al.*, 1998; Rudolph *et al.*, 1999; Kirwan & Dokal, 2008) indicating that telomere dysfunction impairs stem cell function and organ maintenance. Moreover, chronic diseases that accelerate the rate of cell turnover are associated with accelerated telomere shortening and impaired tissue maintenance. Aging-associated diseases of the human hematopoietic system that are associated with critical telomere shortening include myelodysplastic syndromes (MDS) (Keller *et al.*, 2009; Lange *et al.*, 2010) that are characterized by impaired hematopoiesis resulting in low peripheral blood counts and anemia. There

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is an evidence that HSC extrinsic alterations may contribute to disease progression in MDS patients (Marcondes *et al.*, 2009; Mhyre *et al.*, 2009).

Late generation telomerase-deficient mouse *(mTerc$^{-/-}$)* represent a very useful model for probing the molecular mechanisms of telomere driven aging in stem cells. Previous studies have shown that telomere dysfunction induces both cell intrinsic checkpoints and cell extrinsic alterations leading to an age-associated stem cell dysfunction in late generation *mTerc$^{-/-}$* (Choudhury *et al.*, 2007; Ju *et al.*, 2007; Wang *et al.*, 2012). The defects in B-lymphopoiesis and T-lymphopoiesis were strongly associated with cell extrinsic alterations rather than HSC intrinsic checkpoints in telomere dysfunctional mice (Song *et al.*, 2010). However, these defects in differentiation were partially reversible when HSCs were transplanted into an environment with functional telomeres. It has not been investigated in previous studies whether telomere dysfunctional environment could induce stem cell intrinsic defects that impair the self-renewal capacity of HSCs. In addition, it is not known whether environment-induced changes in stem cells would be reversed when stem cell are re-exposed to a young environment.

Here, we show that transient exposure of HSCs to telomere dysfunctional environment induces loss of stem cell quiescence and irreversible intrinsic HSC defects that also persist upon re-transplantation into wild-type environment. By transplantations of hematopoietic stem cells and bones, we show that alterations in the systemic environment are the main cause of impaired HSC quiescence in aging telomere dysfunctional mice. In contrast, alterations in bone-associated stromal niche cells had no measurable influence on HSC aging in the context of telomere dysfunction. These findings suggest that therapeutic targeting of circulating factors could help to slow the induction of cell intrinsic defects impairing HSC maintenance and function.

Results

Transient exposure of hematopoietic stem cells to a telomere dysfunctional environment induces irreversible functional deficiencies

To directly analyze the impact of telomere dysfunctional environment on the evolution of cell intrinsic deficiencies in HSCs, we transplanted wild-type HSCs into lethally irradiated 2- to 3-month-old *G3mTerc$^{-/-}$* mice and age-matched *mTerc$^{+/+}$* control mice (Fig. 1A). Six months after transplantation, the wild-type donor-derived HSCs (Lineage$^-$, Sca-1$^+$, c-Kit$^+$) were re-isolated from *G3mTerc$^{-/-}$* and *mTerc$^{+/+}$* recipient mice by FACS sorting and then transplanted into lethally irradiated wild-type mice in a competitive setting (Fig. 1A). The donor-derived

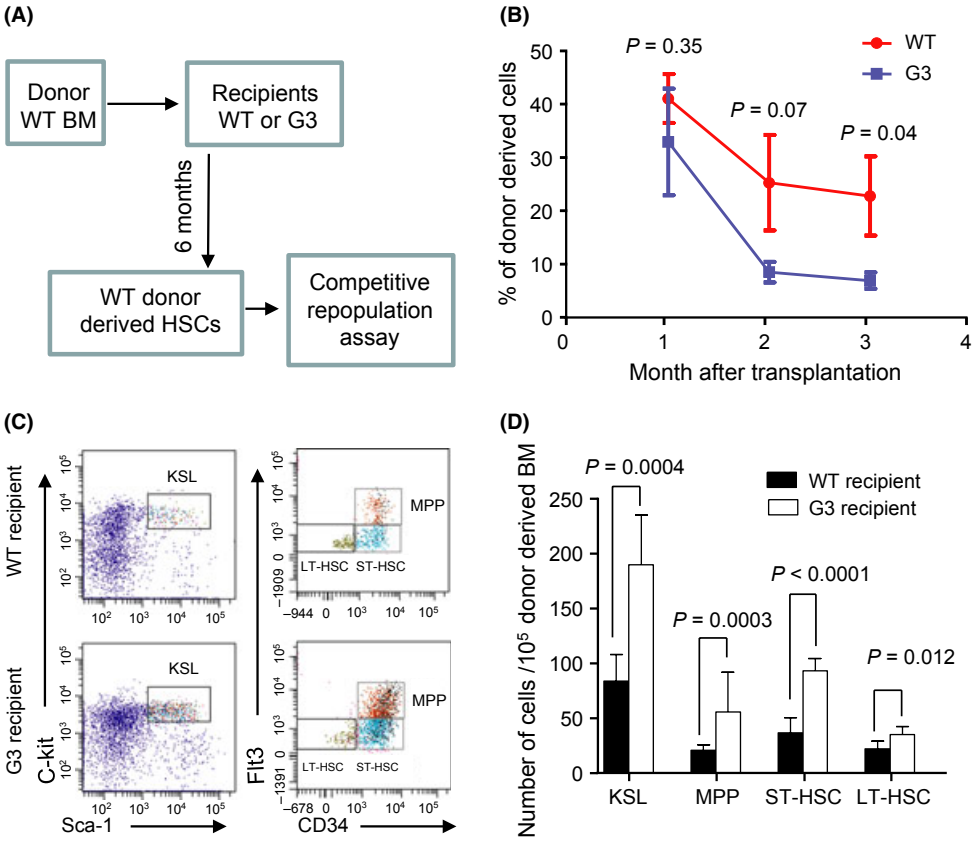

Fig. 1 Increased number but decreased long-term self-renewal of wild-type hematopoietic stem cells (HSCs) when exposed to telomere dysfunctional environment. (A) Experimental scheme shows the wild-type bone marrow cells were transplanted into *G3mTerc$^{-/-}$* and wild-type recipient mice (n = 6 mice per group). Six months after transplantation, the donor-derived HSCs were harvested and follow by secondary transplantation in a competitive setting (n = 6 mice per group). 1000 wild-type donor-derived KSL cells from *G3mTerc$^{-/-}$* or wild-type recipient were transplanted in CD45.1/CD45.2 mice with 2 × 10^5 total bone marrow (CD45.1). (B) Chimerisms of donor-derived peripheral blood cell were measured at indicated time points after the secondary transplantation. Donor cells: WT indicates wild-type donor-derived KSL cells from wild-type recipients; G3 indicates wild-type donor-derived KSL cells from *G3mTerc$^{-/-}$* recipients. (C) Representative FACS profiles show the percentage of wild-type donor-derived KSL (Lineage negative, C-kit positive and Sca-1 positive), LT-HSC (CD34$^-$ Flt3$^-$ KSL), MPP (CD34$^+$, Flt3$^+$ KSL), ST-HSC (CD34$^+$, Flt3$^-$, KSL) in *G3mTerc$^{-/-}$* or wild-type primary recipients. (D) Corresponding bar graph shows the indicated cell number of wild-type donor-derived cell per 10^5 total bone marrow cells in *G3mTerc$^{-/-}$* or wild-type primary recipients.

chimerism in peripheral blood did not show significant difference at 1 month after transplantation, but at 3 months after transplantation, the repopulating capacity of HSCs isolated from *G3mTerc*$^{-/-}$ primary recipients showed significant reduction compared with those isolated from *mTerc*$^{+/+}$ primary recipients (Fig. 1B). The reduced repopulating capacity of wild-type-derived HSCs isolated from *G3mTerc*$^{-/-}$ primary recipients was not associated with a reduction in the number of stem or progenitor cells, but rather correlated with an increased number of immuno-phenotypically defined long-term HSC (LT-HSC), short-term HSC (ST-HSC), and multi-potent progenitors (MPP) in primary recipients (Fig. 1C–D). These data indicate that telomere dysfunction environment induces an expansion of immuno-phenotypically defined stem and progenitor cells, but a net decrease in the long-term repopulating capacity per HSC.

Telomere dysfunctional environment induces a loss of hematopoietic stem cell quiescence

The results on an increase in the number of phenotypic HSCs in response to exposure of HSCs to a telomere dysfunctional environment (Fig. 1C) prompted an analysis of cell cycle profiles because increases in cell proliferation have been associated with expansion in HSC numbers (Cheng *et al.*, 2000; Hock *et al.*, 2004; Wilson *et al.*, 2004). An analysis of the cell cycle profiles of endogenous HSCs in 10-month-old *G3mTerc*$^{-/-}$ and *mTerc*$^{+/+}$ mice (nontransplanted mice) revealed increased proliferation rates in progenitor cells [KSL cells (c-Kit^{+}, Sca-1^{+}, Lineage^{-}), MPPs and ST-HSCs] of *G3mTerc*$^{-/-}$ compared with *mTerc*$^{+/+}$ mice (Fig. 2A–B). Differences were less pronounced on the level of long-term HSCs possibly due to cell intrinsic growth inhibitory effects of dysfunctional telomeres. To directly test the impact of telomere dysfunctional environment on the cell cycle profile of HSCs with long telomere reserves, we transplanted wild-type HSCs into lethally irradiated, 3-month-old *G3mTerc*$^{-/-}$ and *mTerc*$^{+/+}$ mice. The cell cycle profiles of wild-type donor-derived HSCs were analyzed by FACS 6 months after transplantation. Wild-type donor-derived KSL cells, ST-HSCs, and LT-HSCs in *G3mTerc*$^{-/-}$ recipient mice showed significantly increased proliferation compared with those in *mTerc*$^{+/+}$ recipient mice (Fig. 2C–D). These data suggest that telomere dysfunctional environment causes increased proliferation rates of hematopoietic stem and progenitor cells.

Telomere dysfunction in the bone marrow stromal niche does not increase hematopoietic stem cell number or proliferation

To decipher the relative contribution of systemic factors vs. alterations of the hematopoietic niche to the loss of HSC quiescence, we transplanted femoral bones from neonatal *G3mTerc*$^{-/-}$ or *mTerc*$^{+/+}$ mice under the kidney capsule of wild-type mice. Consistent with previous studies, more than 98% of the bone marrow cells in the grafted femur were recipient-derived, whereas the bone-associated stromal niche cells were graft-derived (Song *et al.*, 2010). Eight months after transplantation, the grafted bones were isolated, and the recipient-derived HSCs in these

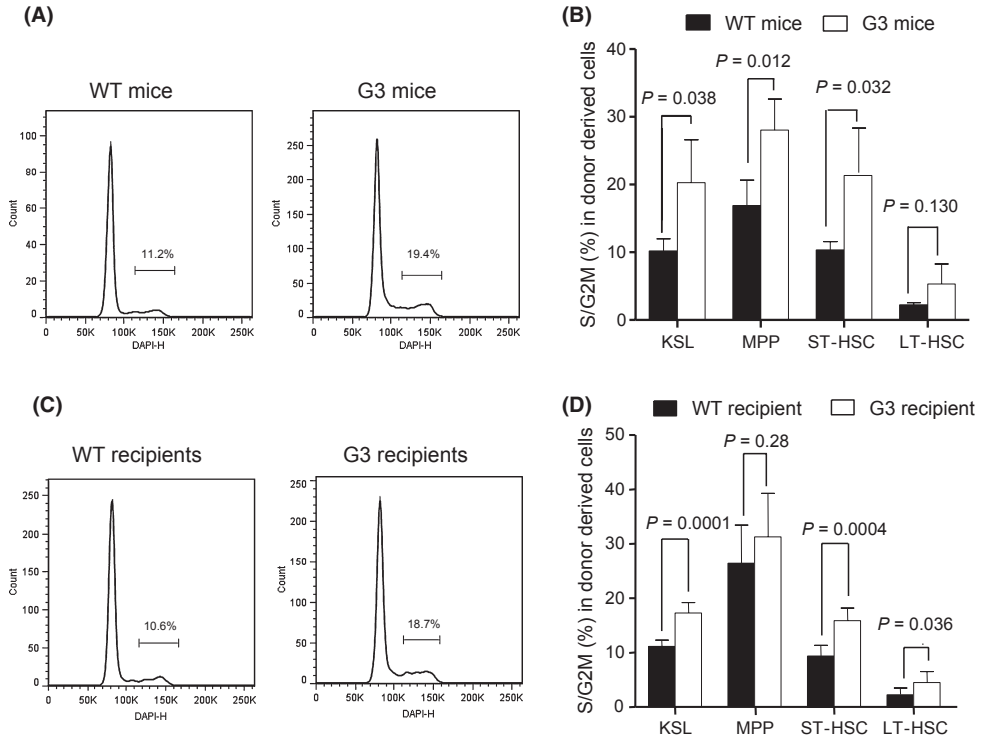

Fig. 2 Loss of hematopoietic stem cell (HSC) quiescence in telomere dysfunctional mice. (A) Representative FACS profiles showing the percentage of cells in S and G2/M phase in endogenous bone marrow KSL cells of *G3mTerc*$^{-/-}$ or wild-type mice. (B) Corresponding bar graph shows the percentage of cell in S and G2/M phase in the endogenous HSCs of *G3mTerc*$^{-/-}$ or wild-type mice (n = 5 mice per group). (C–D) Wild-type stem cells were transplanted into *G3mTerc*$^{-/-}$ and wild-type recipient mice (n = 6 mice per group). Six months after transplantation, the donor-derived HSCs were harvested for cell cycle analysis. (C) Representative FACS profiles showing the percentage of cells in S and G2/M phase in wild-type donor-derived cell from *G3mTerc*$^{-/-}$ or wild-type recipient mice. (D) The corresponding bar graph shows the percentage of cells in S and G2/M phase in donor-derived HSCs from *G3mTerc*$^{-/-}$ or wild-type recipient mice.

bones were analyzed. We did not observe any increase in KSL cells or HSCs cells in *G3mTerc*$^{-/-}$ bones compared with those in *mTerc*$^{+/+}$ bones, but rather a trend of decreased number of HSCs in the *G3mTerc*$^{-/-}$ bones (Fig. 3A–B). Cell cycle analysis of wild-type recipient-derived HSCs in *G3mTerc*$^{-/-}$ or *mTerc*$^{+/+}$ bones showed that the *G3mTerc*$^{-/-}$ bone stromal niche cells had no significant effect on the proliferation of HSCs (Fig. 3C). Next, we isolated bone marrow stromal cells (CD45^{-}Ter119^{-}CD31^{-}7AAD^{-}) from *G3mTerc*$^{-/-}$ and *mTerc*$^{+/+}$ mice, and then cocultured wild-type Lineage^{-} bone marrow cells with these stromal cells. These experiments did not reveal a significant influence of telomere dysfunctional stromal cells on the number of KSL cells or HSCs (Fig. 3D–F). Together, these data indicated that telomere dysfunction of stromal niche cells did not cause the loss of quiescence of HSCs in telomere dysfunction mice.

Telomere dysfunction induced alteration in blood circulatory factors increase hematopoietic stem cell proliferation

Previous experiments indicated that telomere dysfunctional environment limits HSC engraftment by systemic factors including elevated G-CSF serum levels (Ju *et al.*, 2007). To test whether circulatory factors activated HSC proliferation in response to telomere dysfunction, we cultured Lineage^{-} cells from wild-type mice in 2% serum from either *G3mTerc*$^{-/-}$ or *mTerc*$^{+/+}$ mice without additional growth factors. The percentage of KSL cells significantly increased in cultures supplemented with *G3mTerc*$^{-/-}$ serum compared with cultures containing *mTerc*$^{+/+}$ serum (Fig. 4A). This increase in the number of KSL cells was associated with an increased proliferation rate of KSL cell in cultures exposed to *G3mTerc*$^{-/-}$ serum compared with cultures containing *mTerc*$^{+/+}$ serum (Fig. 4B). The apoptosis

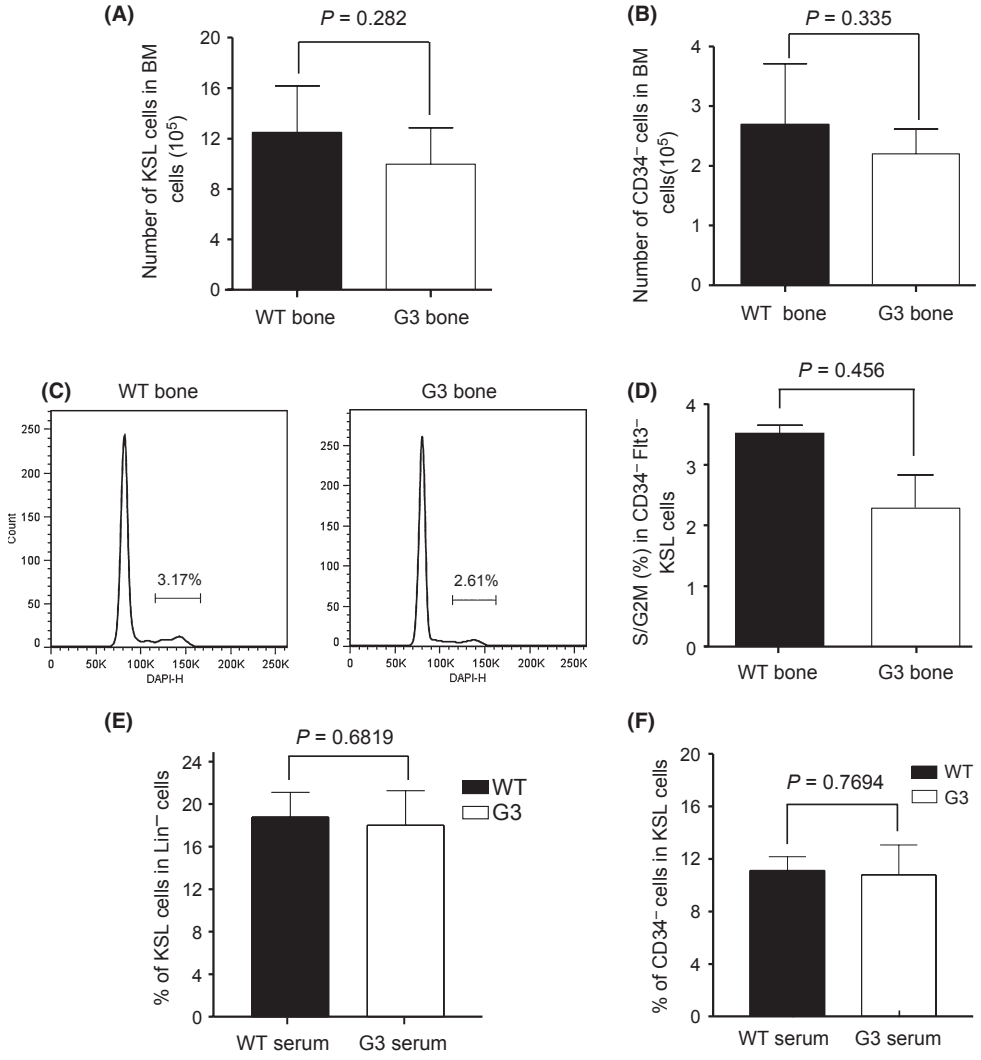

Fig. 3 The increased hematopoietic stem cell (HSC) proliferation was not caused by telomere dysfunctional stromal cells. Wild-type or *G3mTerc*$^{-/-}$ neonatal bone was transplanted into the kidney capsule of wild-type recipient mice (n = 6 mice per group). Eight months after transplantation, the recipient-derived bone marrow cells in the grafted bones were analyzed. The bar graph shows the percentage of (A) KSL and (B) LT-HSC in the bone marrow cells of the grafted bone of indicated genotype. (C) Bar graph shows the percentage of cell in S and G2/M phase in wild-type recipient-derived LT-HSC in the grafted bones. Lineage negative cells from wild-type mice were cultured with stromal cells (CD45-Ter119-CD31-7AAD-) derived from *G3mTerc*$^{-/-}$ or wild-type bone marrow. Thirty-six hours after culture, the cells were harvested and analyzed. The experiment was repeated three times. (D) Bar graph showing the percentage of the KSL cells in Lineage negative cells cultured with stromal cells of indicated genotype (n = 5 mice per group). (E) Bar graph showing the percentage of CD34^{-} Flt3^{-} KSL cells in cultures with stromal cells of indicated genotype. The experiment was repeated three times.

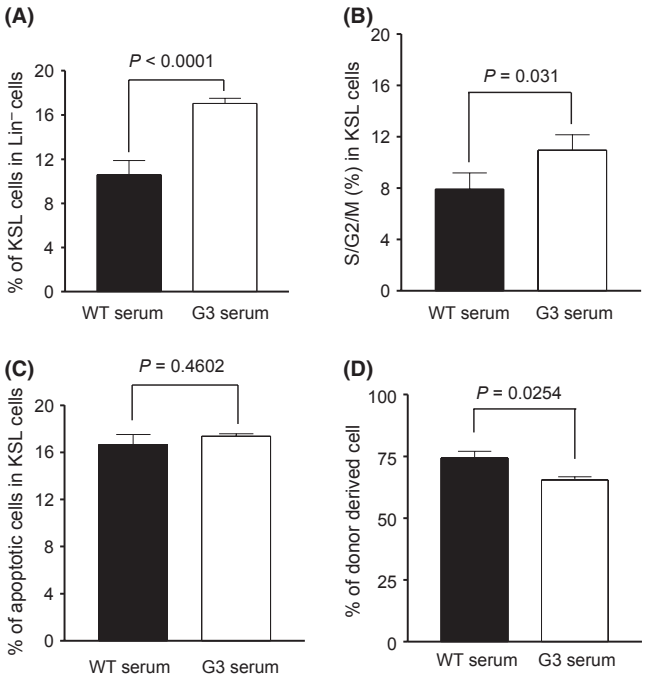

Fig. 4 Increased hematopoietic stem cell (HSC) proliferation was caused by telomere dysfunctional systemic factors. Lineage negative cells from wild-type mice were cultured with wild-type or *G3mTerc*$^{-/-}$ serum. The serum used in this experiment was pooled from 15 mice per group. 36 h after culture, the cells were analyzed (A–C) or transplanted (D) into lethally irradiated young wild-type mice together with competitor cells (n = 6 mice per group). (A) Bar graph shows the percentage of KSL cells in lineage negative cells. The percentage of ki67 positive cells (B) and Annexin V positive cells (C) were showed in KSL cells. (D) Bar graph shows the percentage of donor-derived cells in peripheral granulocytes at 3 months after transplantation. The experiment was repeated twice.

rate of KSL cells cultured with *G3mTerc*$^{-/-}$ serum did not show significant differences compared to those cultured with *mTerc*$^{+/+}$ serum (Fig. 4C). To test impact of short term *in vitro* exposure to *G3mTerc*$^{-/-}$ or *mTerc*$^{+/+}$ serum on the functional reserves of HSCs, we transplanted Lineage^{-} cells from both cultures (36 h after initiating the cultures) in a competitive repopulation assay. Three months after transplantation, the peripheral blood chimerism of test donor cells derived from cultures containing *G3mTerc*$^{-/-}$ serum was significantly reduced compared with test donors derived cells from cultures exposed to *mTerc*$^{+/+}$ serum (Fig. 4D). These data indicated that the impaired HSC quiescence in telomere dysfunctional environment was mainly induced by alteration in blood circulatory factors.

Telomere dysfunction induces multiple factors regulating stem cell function

To understand the molecular mechanisms of loss of quiescence induced by the telomere dysfunctional environment, we examined the gene expression profile of wild-type donor-derived HSCs isolated from *G3mTerc*$^{-/-}$ recipient and *mTerc*$^{+/+}$ recipient mice. The gene expression levels of p21, p27, and TGF-beta1 were significantly lower in wild-type donor-derived HSCs isolated from *G3mTerc*$^{-/-}$ recipient mice compared with those isolated from *mTerc*$^{+/+}$ recipient mice (Fig. S1A–C). Next, we examined the impact of transient exposure to telomere dysfunctional systemic alterations on the gene expression profiles. After 5 h of culture in *G3mTerc*$^{-/-}$ or *mTerc*$^{+/+}$ serum, the gene expression level of p21 and TGF-beta1 were significantly lower in HSCs exposed to *G3mTerc*$^{-/-}$ serum than those exposed to *mTerc*$^{+/+}$ serum (Fig. S1D–E). Moreover, we found that concentration of TGF-beta1 latent form was lower in the serum of *G3mTerc*$^{-/-}$ mice compared with age-matched *mTerc*$^{+/+}$ mice (Fig. S1F). To further dissect the potential contribution of serological TGF-beta1 to the loss of HSC quiescence induced by telomere dysfunctional environment, we block the TGF-beta1 active form by neutralizing antibody in cultures supplied with *G3mTerc*$^{-/-}$ serum or *mTerc*$^{+/+}$ serum. Blocking of TGF-beta1 active form increased the percentage of KSL cells not only in cultures supplied with *mTerc*$^{+/+}$ serum but also in those supplied with *G3mTerc*$^{-/-}$ serum (Fig. S2A). Furthermore, we examined the potential contribution of p21 to the loss of quiescence in *ex vivo* cultures exposed to *G3mTerc*$^{-/-}$ or *mTerc*$^{+/+}$ serum. The proliferation of p21$^{-/-}$ KSL cells was higher compared with p21$^{+/+}$ KSL cells not only in cultures supplied with *mTerc*$^{+/+}$ serum but also in those supplied with *G3mTerc*$^{-/-}$ serum (Fig. S2B). These data suggested that telomere dysfunction induces a complex network involving multiple circulating factors and cell intrinsic alterations that promote HSC immuno-phenotypic expansion but functional exhaustion. A combined investigation of these factors could help to better understand the mechanism of stem cell exhaustion in aged tissue compartments with elevated rates of DNA damage.

Discussion

The current study shows that telomere dysfunction induces alterations in the stem cell environment that lead to HSC intrinsic deficiencies that are maintained upon transplantation. These results provide the first proof of concept that stem cell intrinsic defects can be induced by environmental alterations. Previous studies have revealed multiple evidences that both cell intrinsic and cell extrinsic mechanisms can contribute to a decline in stem cell function with aging. However, a cross-talk between extrinsic and intrinsic mechanisms of stem cell aging has not been provided. In fact, it was reported that defects in stem cell function (biased lineage commitment) induced by the environment are partially reversible when stem cells are re-exposed to a young environment (Conboy *et al.*, 2005) or to an environment with long telomere reserves (Ju *et al.*, 2007; Song *et al.*, 2010). The current data indicate that environmental alterations can induce stem cell intrinsic defects (reduced long-term repopulating capacity) that persist even after re-exposing stem cells to a functional environment. These findings could have implication for therapeutic approaches aiming to improve the function of aged stem cells to improve organ maintenance.

The exact nature of stem cell intrinsic defects that are induced by the environment remains yet to be determined. Our data show an association between environment-induced reductions in HSC function and increased rates of stem cell proliferation. In addition, the loss of HSC quiescence in response to telomere dysfunction is mainly induced by alteration in the systemic environment. Transplantation of HSCs and bones showed that the impairment in HSC function was associated with the telomere status of the recipient mice, whereas telomere dysfunction in bone-associated stromal cells had no significant effects on the loss of HSC quiescence in late generation telomerase knockout mice.

Together, the current study revealed the first experimental evidence that telomere dysfunction induces alteration in the systemic environment that lead to a loss of HSC quiescence resulting in stem cell intrinsic defects that cannot be rescued after transplantation of HSCs into a wild-type environment. The systemic factors that impair HSC function in response to telomere dysfunction induced changes in the environment remain yet to be defined. It has been shown that telomere dysfunction in telomerase

knockout mice induces the expression of marker proteins of DNA damage in blood serum (Jiang *et al.*, 2008). Recent studies showed that senescence changes the secretome of cells exhibiting cell proliferation stimulating activity (Coppe *et al.*, 2008). It is conceivable that similar (Allsopp *et al.*, 1992) mechanisms could lead to an increase in HSCs proliferation. A systemic analysis of these regulatory networks could help to identify novel targets for therapies aiming to improve stem cell function during aging.

Materials and methods

Mice

Mice were maintained in a pathogen-free environment and fed with a standard diet. C57BL/6 congenic mice expressing CD45.1 on leukocytes were used for transplantation experiments. Third-generation telomerase-deficient mice (*G3mTerc*$^{-/-}$) or *mTerc*$^{+/+}$ mice (CD45.2) were used as recipient mice, and 2- to 3-month-old wild-type mice (CD45.1) were used as donor for bone marrow transplantation. Neonatal bones were isolated from *G3mTerc*$^{-/-}$ or *mTerc*$^{+/+}$ mice were transplanted under the kidney capsule of 2- to 3-month-old *G3mTerc*$^{-/-}$ or *mTerc*$^{+/+}$ mice side by side. All animal experiments were approved by the state government of Baden-Württemberg.

Bone transplantations

Survival surgery was performed under sterile conditions after intraperitoneal administration of the anesthetic, ketamine (100 mg kg^{-1}) and xylazine (10 mg kg^{-1}) to 2- to 3-month-old *G3mTerc*$^{-/-}$ or *mTerc*$^{+/+}$ recipient mice. A small dorsolateral incision was made to expose the kidney and a small hole was made in the kidney capsule. Neonatal hindlimb bones from *G3mTerc*$^{-/-}$ or *mTerc*$^{+/+}$ donor were placed under the kidney capsule side by side in recipient mice. The grafted bones were analyzed 8 months after transplantation.

Bone marrow transplantation

Bone marrow cells (2×10^6) from 2- to 3-month-old wild-type mice (CD45.1) were transplanted into lethally irradiated *G3mTerc*$^{-/-}$ or *mTerc*$^{+/+}$ (CD45.2) mice. Six months after transplantation, the 1000 wild-type donor (CD45.1)-derived KSL (Lineage$^-$, Sca-1$^+$, c-Kit$^+$) cells were isolated from *G3mTerc*$^{-/-}$ and *mTerc*$^{+/+}$ recipient mice by FACS sorting, and then transplanted into lethally irradiated wild-type mice (CD45.1/CD45.2) together with 2×10^5 total bone marrow cells (CD45.1) as competitors. The donor-derived (CD45.1) chimerisms in peripheral blood were tested at indicated time point.

Flow cytometry and cell cycle analysis

Bone marrow cells were isolated by flushing both tibias and femurs with sterile PBS. Cells were filtered and counted before staining with antibodies. For cell cycle analysis, total bone marrow cells were stained with stem cell surface markers (Lineage, Sca-1, c-kit, CD34), then fixed and permeabilized with BD Cytofix/Cytoperm Buffer, followed by staining with ki67 antibody and DAPI. Data acquisition was performed on FACS LSRII, cell sorting were performed on FACS Aria II. Data were analyzed on the Diva6.1 software.

Enzyme-linked immunosorbent assay

The experiment was performed using Mouse TGF-beta1 Enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA). Briefly, the serum of wild-type and *G3mTerc*$^{-/-}$ was activated by NHCL. 50 μL of Assay Diluent RD1-73 and 50 μL of activated sample was added into each well, incubate for 2 h at room temperature. After washing, 100 μL of TGF-β1 conjugate was added into each well and incubate for 2 h at room temperature. After washing, 100 μL of substrate solution was added into each well and incubate for 30 min at room temperature. Finally, 100 μL of stop solution was added into each well, followed by analysis in a microplate reader set at 450 nm.

In vitro culture

Lineage negative cells (1×10^6) from wild-type mice (CD45.1) were cultured in 1 mL StemSpan® Serum-Free Expansion Medium (SFEM) supplied with 2% serum from wild-type or *G3mTerc*$^{-/-}$ mice. After 36 h in culture, cells were collected and used for competitive transplantation. For each lethally irradiated recipient (CD45.2), 1×10^6 total cultured cells (CD45.1) and 1×10^6 total bone marrow cells (CD45.1/CD45.2) were transplanted. The donor-derived chimerisms in peripheral blood were examined at indicated time point.

Statistics

We carried out statistical analysis using Microsoft Excel and Graph Pad Prism software. The unpaired Student's *t*-test was used to generate *P*-values for all the datasets. Error bars represent SD in all figures.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Fig. S1 Gene expression profile of wild-type HSCs exposed to telomere dysfunctional environment.

Fig. S2 Repression of TGF-b1 or p21 alone cannot reverse the loss of HSC quiescence induced by telomere dysfunctional environment.

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