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Brief Report: Long-Term Functional Engraftment of Mesenchymal Progenitor Cells in a Mouse Model of Accelerated Aging

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ABSTRACT

Age-related osteoporosis is characterized by a decrease in bone-forming capacity mediated by defects in the number and function of osteoblasts. An important cellular mechanism that may in part explain osteoblast dysfunction that occurs with aging is senescence of mesenchymal progenitor cells (MPCs). In the telomere-based $Wrn^{-/-}Terc^{-/-}$ model of accelerated aging, the osteoporotic phenotype of these mice is also associated with a major decline in MPC differentiation into osteoblasts. To investigate the role of MPC aging as a cell-autonomous mechanism in senile bone loss, transplantation of young wild-type whole bone marrow into $Wrn^{-/-}Terc^{-/-}$ mutants was performed and the ability of engrafted cells to differentiate into cells of the osteoblast lineage was assessed. We found that whole bone marrow transplantation in $Wrn^{-/-}Terc^{-/-}$ mice resulted in functional engraftment of MPCs up to 42 weeks, which was accompanied by a survival advantage as well as delays in microarchitectural features of skeletal aging. STEM CELLS *2013;31:607–611*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

Skeletal aging of bone is characterized by loss of mineral content and microarchitectural changes that decrease bone strength and predispose bone to damage from repeated loading [1, 2].

Osteoporosis is common in the Werner and dyskeratosis congenita premature aging syndromes, both characterized by telomere dysfunction [3, 4]. One of the targets of WRN helicase is telomeric DNA, but need for WRN at telomeres is minimized in mice by long telomeres and abundant telomerase, making Wrn knockout mice relatively unaffected [5, 6]. However, combining Wrn mutation with shortened telomeres of telomerase (Terc) knockout mice results in an accelerated aging model [5, 7]. Deficiencies in $Wrn^{-/-}Terc^{-/-}$ mutant mice cause a low bone mass phenotype due to impaired osteoblast differentiation in the context of intact osteoclast differentiation [8, 9]. This impaired differentiation is associated with telomere dysfunction, as measured by the association of DNA damage proteins with telomeres in mesenchymal progenitor cells (MPCs) isolated from double mutant mice [9]. MPCs from $Wrn^{-/-}Terc^{-/-}$ mutants have a reduced in vitro lifespan but also display impaired osteogenic potential with dysfunctional telomeres independently of proliferative state [9]. Here, we test the hypothesis that MPC aging contributes to bone loss in an accelerated aging mouse model that recapitulates many aspects of age-related bone loss.

MATERIALS AND METHODS

Detailed Materials and Methods are described in supporting information.

Author contributions: L.S., T.B., J.-H.K., and Y.Z.: collection and/or assembly of data, data analysis and interpretation, manuscript writing, and final approval of manuscript; K.P.E. and E.A.M.: collection and/or assembly of data and final approval of manuscript; Q.C.: collection and/or assembly of data, provision of study materials, and final approval of manuscript; K.D.H. and S.G.E.: data analysis and interpretation, manuscript writing, and final approval of manuscript; F.B.J.: collection and/or assembly of data, provision of study materials, data analysis and interpretation, manuscript writing, and final approval of manuscript; R.J.P.: conception and design, financial support, collection and/or assembly of data, data analysis and interpretation, manuscript writing, and final approval of manuscript. L.S. and T.A.B. contributed equally to this article.

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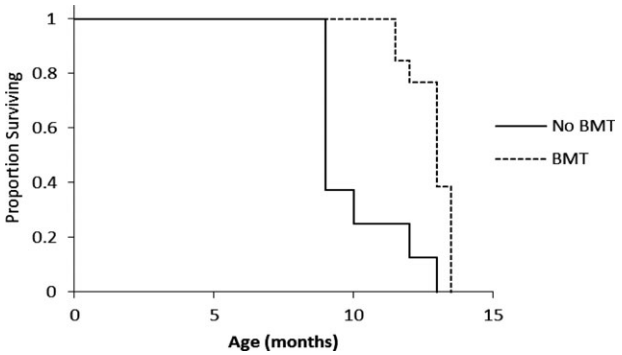

Figure 1. Overall survival advantage of $Wrn^{-/-}Terc^{-/-}$ mice after BMT. Kaplan-Meier plot of $Wrn^{-/-}Terc^{-/-}$ mutants with ($n = 13$) and without ($n = 8$) wild-type whole BMT. $p = .00035$ by log-rank test. Abbreviation: BMT, bone marrow transplantation.

Results

Wild-Type Whole Bone Marrow Transplantation into $Wrn^{-/-}Terc^{-/-}$ Mutants Confers a Survival Advantage

To test the role of telomere-based MPC aging on age-related osteoporosis, $Wrn^{-/-}Terc^{-/-}$ mutants were transplanted at 3 months of age with whole bone marrow (BM) from young wild-type donors. At 10.5 months after transplantation or when animals exhibited signs of significant distress and impending demise (whichever occurred first), mutants were sacrificed for analysis of functional MPC engraftment and concomitant measurements of skeletal microarchitectural features. As shown in Figure 1, transplanted animals exhibited a survival advantage, having a mean life span approximately 30% longer than untransplanted controls (12.89 ± 0.21 months vs. 10 ± 0.57 months). This difference is particularly remarkable given that telomerase-deficient mice are hypersensitive to ionizing radiation and otherwise would have been expected to incur substantial harm from irradiation associated with bone marrow transplantation (BMT) [10].

Long-Term Functional Engraftment of MPCs in $Wrn^{-/-}Terc^{-/-}$ Mice

Enhanced green fluorescent protein-positive (GFP+) wild-type mice were used as donors in all BMT experiments. GFP+ MPCs in BM aspirates were identified from transplanted animals by fluorescent immunohistochemistry and represented 54.0% ± 7.1% of plastic adherent stromal cells after 30 hours in culture. Expanded MPC cultures from young wild-type donor animals are essentially $CD45^-$ $Sca\text{-}1^+$ cells and can differentiate in vitro into osteoblasts and adipocytes (supporting information Fig. S1). $CD45^-$ $Sca\text{-}1^+$ MPCs are present after long-term BMT in $Wrn^{-/-}Terc^{-/-}$ mice (Fig. 2).

MPCs demonstrate functional engraftment as differentiated GFP^+ osteoblasts and osteocytes in bone sections from recipient $Wrn^{-/-}Terc^{-/-}$ mice (Fig. 3, supporting information Fig. S2). MPC functional engraftment is present up to 10.5 months after BMT. In femur sections from transplanted animals, 20% ± 8% of cortical osteocytes and 6% ± 2% of trabecular osteocytes were derived from engrafted precursors. Among endocortical and trabecular bone-lining osteoblasts, 15% ± 6% and 5% ± 1% were from precursors of donor origin, respectively. Differentiation of engrafted MPCs was most evident as endocortical osteocytes (supporting information Fig. S2).

Delays in Microarchitectural Features of Skeletal Aging in $Wrn^{-/-}Terc^{-/-}$ Mutants After BMT

Despite being ~30% older than nontransplanted double mutants, $Wrn^{-/-}Terc^{-/-}$ BMT recipients had preserved or improved measures of bone microarchitecture. Transplanted animals showed no statistically significant changes in

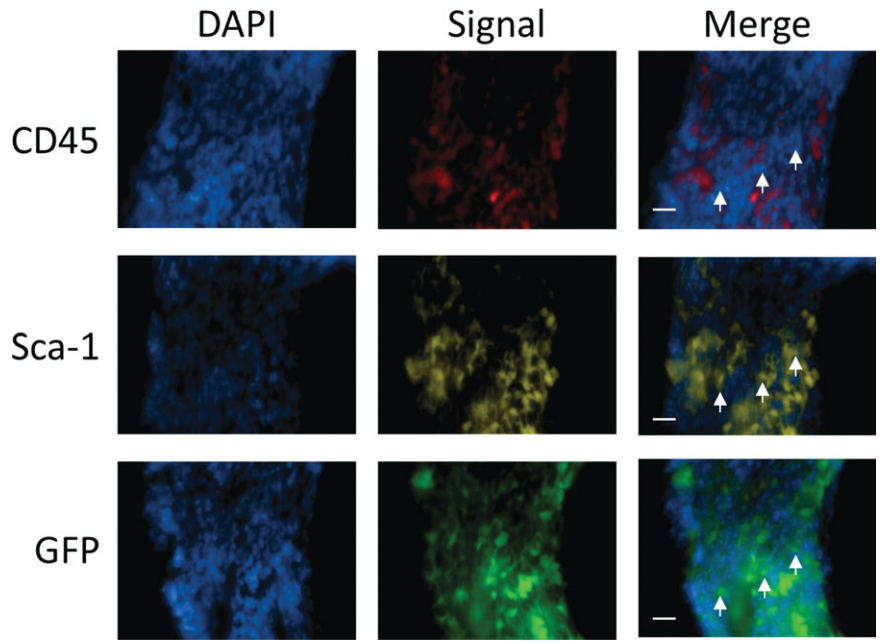

Figure 2. Long-term engraftment of $CD45^-Sca\text{-}1^+$ mesenchymal progenitor cells (MPCs) after bone marrow transplantation. Serial bone sections were stained with the indicated antibodies at left. Representative examples of engrafted MPCs in a $Wrn^{-/-}Terc^{-/-}$ recipient are shown by arrows. Scale bar = 20 μm. Abbreviations: DAPI, 4',6-diamidino-2-phenylindole; GFP, green fluorescent protein.

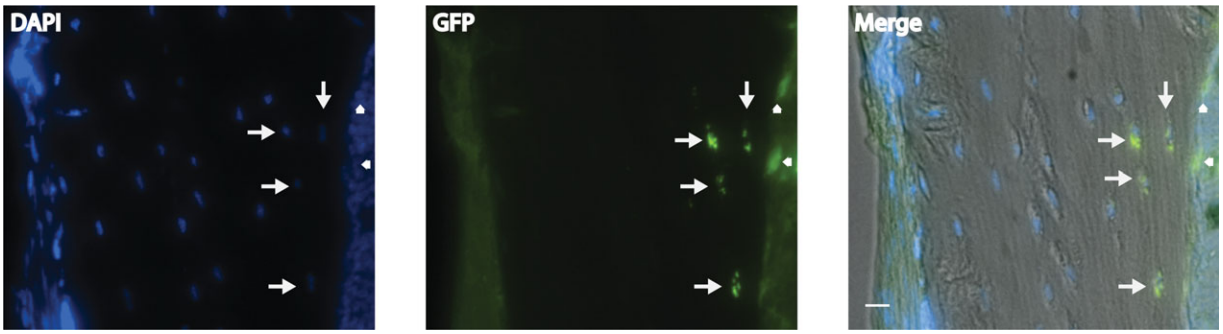

Figure 3. Donor mesenchymal progenitor cells differentiate into bone-lining osteoblasts (and subsequently osteocytes) that are incorporated into bone. Arrowheads indicate bone-lining cells. Arrows indicate osteocytes located in their lacunae. Scale bar = 10 μm. Abbreviations: DAPI, 4',6-diamidino-2-phenylindole; GFP, green fluorescent protein.

trabecular bone volume/total volume, trabecular number, or in cortical thickness (Fig. 4). Interestingly, there was a statistically significant increase in the ratio of cortical area to total area, suggesting that transplanted mice were able to improve endocortical bone mass. Similarly, $Wrn^{-/-}Terc^{-/-}$ BMT recipients had preserved osteoblasts/bone surface and no increase in the number of osteoclasts/bone surface (supporting information Fig. S3).

Discussion

Although the causal role(s) of telomere dysfunction in age-related osteoporosis are not completely established, there is evidence of its importance. Telomere lengthening mechanisms are not present in human BM MPCs [11]. Exogenous telomerase expression extends in vitro proliferative capacity, accelerates osteogenic differentiation, and enhances bone formation upon subcutaneous transplantation into mice [12, 13]. In addition, progeroid syndromes on which the $Wrn^{-/-}Terc^{-/-}$ mutants are based (Werner syndrome and dyskerostis congenita, respectively) display premature osteoporosis [3, 4].

Although we cannot exclude that hematopoietic precursors including hematopoietic stem cells (HSCs), transplanted along with MPCs, may support mesenchymal engraftment and/or differentiation into cells of the osteoblast lineage, it is unlikely that $CD45^{-}$ $Sca\text{-}1^{+}$ MPCs are derived directly from HSCs. However, there may be an early common BM progenitor for hematopoietic cells and osteoblasts delineated as $Lin^{-}Sca\text{-}1^{+}cKit^{+}CD45^{+}$ [14, 15].

Soluble hematopoietic factors alone may be sufficient to exert effects on bone remodeling, or development of osteoblasts may depend on the proximity of hematopoietic cells. Sca-1, a cell-surface molecule also expressed on HSCs, appears to be necessary to maintain self-renewal of MPCs and suggests that the latter is plausible. In support of this, Sca-1 knockout mice develop age-dependent osteoporosis [16].

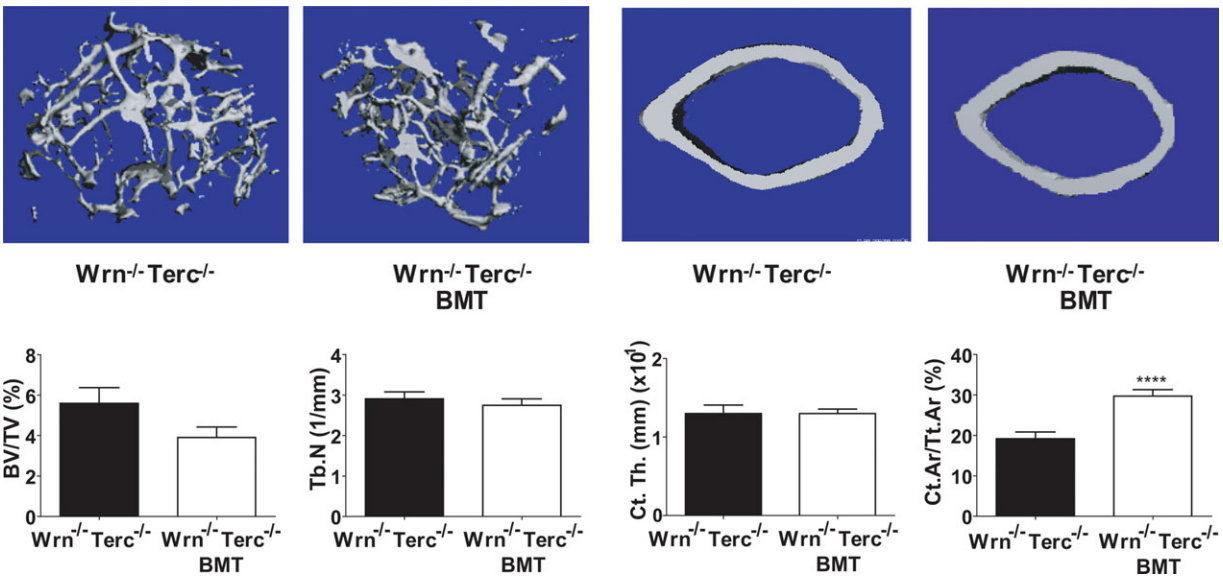

Figure 4. Microarchitectural features of skeletal aging are delayed in $Wrn^{-/-}Terc^{-/-}$ mutants after BMT. (Top panels) Representative micro-CT 3-dimentional reconstructions of trabecular and cortical bone in $Wrn^{-/-}Terc^{-/-}$ mutants without ($n = 6$) and with BMT ($n = 6$) are shown. (Bottom panels) Quantification of trabecular and cortical bone parameters in $Wrn^{-/-}Terc^{-/-}$ mutants demonstrates preservation of microarchitectural features with BMT. Note that BMT recipients were approximately 30% older than non-BMT controls. ****, $p < .002$. Abbreviations: BMT, bone marrow transplantation; BV, bone volume; Ct Th, cortical thickness; Ct.Ar; cortical area; TV, total volume; Tb.N, trabecular number; Tt.Ar, total area.

There could be systemic effects of BMT which may influence skeletal engraftment and differentiation of MPCs. Also, microenvironmental factors (including oxidative stress) may be at play. For example, we previously showed that osteoblast differentiation of MPCs from $Wrn^{-/-}Terc^{-/-}$ mutants is rescued by reducing oxidative stress [9]. Increased oxidative stress favoring senescence in BM MPCs has also been postulated based on proteome screening of these cells from young and old rodents [17]. Muscle-derived stem/progenitor cells from young wild-type mice transplanted into a murine progeria model extended life span and improved degenerative changes in tissues where donor cells are not detected [18]. Although we cannot be sure that extraskeletal effects of BMT are responsible for life span extension in $Wrn^{-/-}Terc^{-/-}$ mutants, the fact that $GFP^{+}Sca\text{-}1^{+}CD45^{-}$ MPCs are present in bone tissue after long-term transplantation suggests that they play a role in maintenance of bone microarchitectural features over the period of extended survival.

To the extent that bone loss with physiologic aging involves telomere-based aging, our data indicate MPC senescence is a contributory mechanism. However, BMT as a therapeutic strategy may be limited by inadequate MPC engraftment [19]. It is debatable whether donor MPCs from human or mouse sources have sustained engraftment in host BM; however, our and other reports indicate that this is so [20–26]. Therefore, it is reasonable to suggest that decreased bone regeneration with age may be partially reversed by transplantation of young donor MPCs.

$Wrn^{-/-}Terc^{-/-}$ mutants may have altered BM stroma which permits effective engraftment of donor MPCs. Thus BMT, performed for other reasons in telomere-based accelerated aging syndromes (e.g., aplastic anemia), may also confer beneficial effects in stabilizing or delaying premature osteoporosis. In fact, aplastic anemia in individuals with telomere-based progeroid syndromes may actually occur due to defective (telomerase-deficient) stroma which cannot support HSCs. Whole BMT in these patients may be successful [27] because it theoretically will correct both the stromal as well as the hematopoietic defects.

Conclusions

Replacement of aging MPCs with young cells results in delays or amelioration of aspects of skeletal aging. $Wrn^{-/-}Terc^{-/-}$ recipients of whole BMT have functional reconstitution of MPCs and stable or improved bone microarchitectural features compared to much younger, nontransplanted mice.

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Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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