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Ameliorating replicative senescence of human bone marrow stromal cells by PSMB5 overexpression

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ABSTRACT

Multipotent human bone marrow stromal cells (hBMSCs) potentially serve as a source for cell-based therapy in regenerative medicine. However, *in vitro* expansion was inescapably accompanied with cell senescence, characterized by inhibited proliferation and compromised pluripotency. We have previously demonstrated that this aging process is closely associated with reduced 20S proteasomal activity, with down-regulation of rate-limiting catalytic β-subunits particularly evident. In the present study, we confirmed that proteasomal activity directly contributes to senescence of hBMSCs, which could be reversed by overexpression of the β5-subunit (PSMB5). Knocking down PSMB5 led to decreased proteasomal activity concurrent with reduced cell proliferation in early-stage hBMSCs, which is similar to the senescent phenotype observed in late-stage cells. In contrast, overexpressing PSMB5 in late-stage cells efficiently restored the normal activity of 20S proteasomes and promoted cell growth, possibly via upregulating the Cyclin D1/CDK4 complex. Additionally, PSMB5 could enhance cell resistance to oxidative stress, as evidenced by the increased cell survival upon exposing senescent hBMSCs to hydrogen peroxide. Furthermore, PSMB5 overexpression retained the pluripotency of late-stage hBMSCs by facilitating their neural differentiation both *in vitro* and *in vivo*. Collectively, our work reveals a critical role of PSMB5 in 20S proteasome-mediated protection against replicative senescence, pointing to a possible strategy for maintaining the integrity of culture-expanded hBMSCs by manipulating the expression of PSMB5.

1. Introduction

Following appropriate induction, multipotent human bone marrow stromal cells (hBMSCs) can differentiate into various lineages including neuronal cells, osteoblasts, adipocytes and chondroblasts, enabling their potential clinical application in transplantation [1–3]. However, after *in vitro* expansion, hBMSCs progressively exhibit replicative senescence which is characterized by impaired ability to differentiate and proliferate, and decreased viability upon exposure to environmental stress [4,5]. This rapid aging process has precluded their utilization in cell-based regenerative medicine and tissue engineering.

The proteasomal pathway plays a pivotal role in maintaining cellular homeostasis through degrading proteins that are unfolded, disordered or damaged, or even redundant [6]. The mammalian proteasome exists as a 26S protease complex comprising one 20S core and two 19S regulatory caps [6]. The 20S core that determines the overall catalytic activity is further composed of multiple α- and β- subunits [7,8]. The β5 (PSMB5) acts as the step-limiting regulator in the process of proteasome-mediated protein degradation [9], and α- subunits are observed to be superfluous and non-integrated in senescent cells [10]. Proteasomal dysfunction is possibly associated with many cellular and biological processes related to cell activity and vitality [9]. For example, reduced proteasomal activity in aged human T cells potentially leads to immune senescence [9,11]. Likewise, the compromised proteasomal function and lowered PSMB5 expression are also found in embryonic fibroblasts with multiple passages [9,10]. We have previously demonstrated that 20S proteasomal activity is critical for retaining stem cell

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integrity of hBMSCs, where enhancement of proteasomal activity by 18α-glycyrrhetinic acid (18α-GA) helps counteract replicative senescence [12].

The strategy to restore stem cell function by overexpressing certain transcription factors has been highly successful in inducing pluripotent stem cells [13]. In addition to transcription factor-mediated genomic remodeling, other manipulations that maintain cellular homeostasis may also be instrumental in protecting cells against aging. Of note, prior studies which correlate the senescence-like phenotype in cultured cells with their partial loss of proteasomal activity have reported improvement following overexpression of the subunit β5 [9,10,14,15]. Given the crucial role of PSMB5 in facilitating the efficient formation of functional proteasome [10], we investigate the impact of PSMB5 overexpression on enhancement of proteasomal activity and maintenance of normal pluripotency in senescent hBMSCs using both *in vitro* and *in vivo* models.

2. Materials and methods

2.1. Cellular model and animals

Human bone marrow stromal cells (hBMSCs) and BALB/c mice were obtained from ScienCell Research Laboratory (San Diego, CA) [12] and the Research Animal Center of Shanxi Medical University, respectively. All experimental procedures were approved by Shanxi Animal Research Ethics Committee.

2.2. Constructs and lentiviral production

The full-length PSMB5 cDNA was a gift from Dr. Murata [16]. Both the overexpression and knockdown constructs of PSMB5 with the lentiviral backbone were designed and produced by Genechem Company (Shanghai, China). Viral packaging, titering and infection were performed following the standard protocols [17,18] (see Suppl. information).

2.3. Western-blotting and real-time PCR assay

Procedures for Western-blotting and real-time PCR were performed as previously described [12]. Antibodies and primer sequences used in the current study were listed in Suppl. Tables 1 and 2, respectively.

2.4. 20S proteasomal activity assay

As reported before [12], protein lysates were prepared and proteasomal activity was determined with the Proteasome Activity Assay Kit (Millipore, Billerica, MA).

2.5. BrdU incorporation and senescence-associated β-galactosidase staining assay

Cell proliferation and replicative senescence were measured by the BrdU incorporation assay and SA-β-gal staining, respectively, as previously mentioned [12].

2.6. Assessment of reactive oxygen species and cell survival in response to oxidative stress

Intracellular levels of reactive oxygen species (ROS) were quantified with the oxidant-sensitive probe 2,7-dichlorofluorescin diacetate (DCFH-DA, Sigma–Aldrich, St. Louis, MO). After hydrogen peroxide (H_2O_2) treatments, the survival of cells was determined by the CCK-8 assay (Dojindo Laboratories, Kumamoto, Japan; see Suppl. information).

2.7. Transplantation of hBMSCs into the mouse brain

Neural induction was performed as previously addressed [3]. After pre-differentiation, cells were injected into the mouse cortex. Antibodies used for immunochemical analysis were listed in Suppl. Table 1.

2.8. Statistical analysis

Data are presented as mean ± SEM. Student's *t*-test and one-way ANOVA were applied to compare 2 or ⩾3 sets of data, respectively. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Restored proteasomal activity in late-stage hBMSCs following PSMB5 overexpression

Aging- and disease-related loss of proteasomal function has been documented by different groups [19,20]. Our previous study suggested that replicative senescence of hBMSCs may be due to decreased proteasomal activity following down-regulation of the rate-limiting subunit PSMB5 [12]. In this work, we first found that knocking down PSMB5 in early-stage hBMSCs significantly reduced proteasomal activity (Suppl. Fig. 1A and B) and decreased cell proliferation (Suppl. Fig. 1C), which again indicated the crucial role of PSMB5-dependent proteasomal activity in this aging process. Using the lentivirus-based system, the expression levels of both the PSMB5 protein (∼2.5-fold vs. GFP control; Fig. 1Aa and b) and its messenger RNA (>5-fold vs. GFP control; Fig. 1B) were substantially raised 5 days after viral infection in late-stage cells without affecting the expression of other subunits, such as PSMB1, PSMB2 or PSMB6 (Fig. 1B). Following PSMB5 overexpression, the proteasomal activity of late-stage hBMSCs also increased significantly from 58% to 79% of that of the early-stage cells ($p < 0.01$ vs. GFP control; Fig. 1C). These data demonstrated that we managed to efficiently overexpress PSMB5, which restored the proteasomal activity in late-stage hBMSCs.

3.2. PSMB5 overexpression promoted cell proliferation in late-stage hBMSCs by upregulating cell cycle-related proteins

Retarded proliferation has been reported in hBMSCs after prolonged culture expansion [4,5]. In the current study, we observed that the percentage of BrdU-positive ($BrdU^+$) hBMSCs in the late stage was dramatically decreased by 41% compared with that in the early stage (Fig. 2A). Given that the Cyclin D1/CDK4 complex accumulation is the putative indispensable step in cell cycle progression [21], we next examined whether this protein complex is related to the decreased cell proliferation in late-stage hBMSCs. As expected, the protein levels of Cyclin D1 and CDK4 were substantially lowered in senescent hBMSCs by ∼60% and ∼80%, respectively ($p < 0.01$ vs. Early-stage controls; Fig. 2A). In contrast, as shown in Fig. 2B, the number of $BrdU^+$ cells was significantly increased by ∼14% following PSMB5 overexpression ($p < 0.01$ vs. GFP control). Meanwhile, overexpressing PSMB5 attenuated the reduction of Cyclin D1 and CDK4 proteins, raising their expressions by 56% and 23%, respectively (Fig. 2B). These observations demonstrated that PSMB5 overexpression could rejuvenate senescent hBMSCs by enhancing their proliferation, which potentially

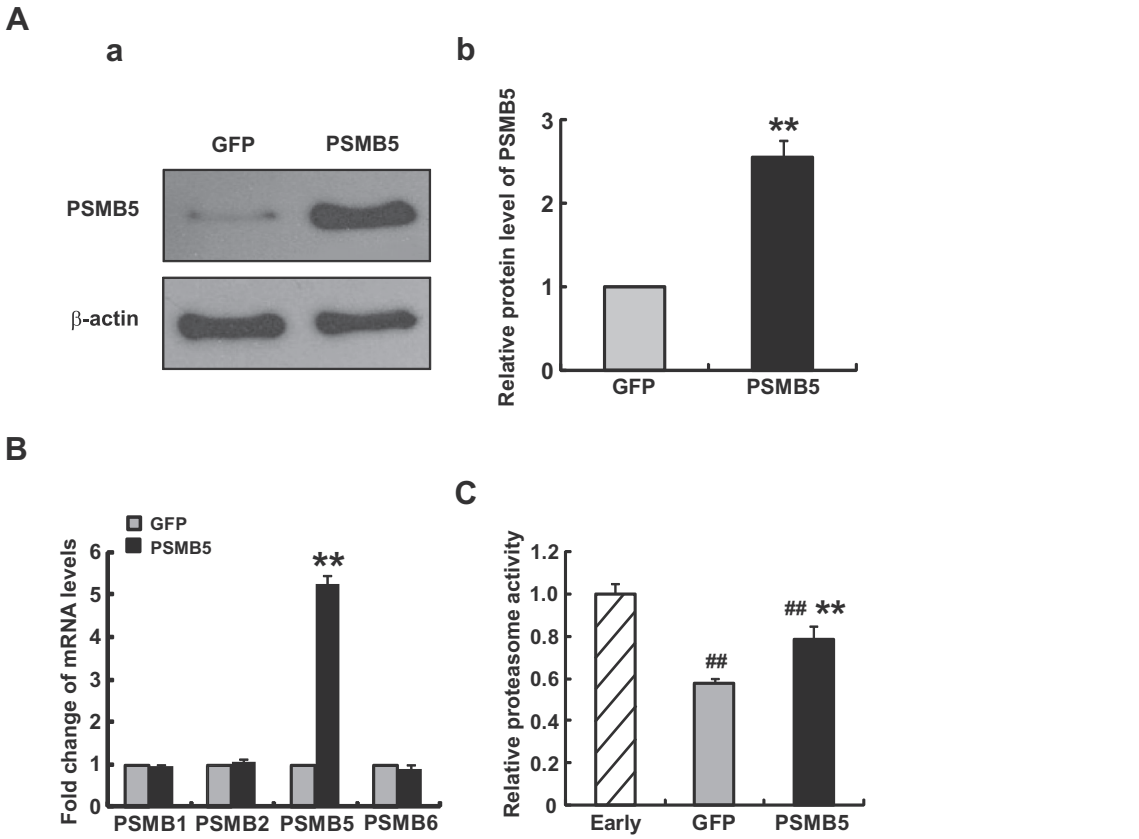

Fig. 1. Restored proteasomal activity in the late-stage hBMSCs following PSMB5 overexpression. (A) (a) Introduction of PSMB5 into late-stage hBMSCs increased the protein level of PSMB5. (b) The quantitative data of (a). (B) Real-time PCR was used to examine the mRNA levels of β-subunits after viral infection. (C) 20S proteasomal activity was rescued after PSMB5 overexpression, comparable to that in early-stage cells. Data were obtained from three independent experiments. $^{\#\#}p < 0.01$ vs. Early-stage cells; $^{**}p < 0.01$ vs. GFP control.

depended on the upregulation of the cell-cycle regulator Cyclin D1/CDK4 complex that assists in the G1 to S phase transition.

3.3. Delayed replicative senescence and promoted cell survival under oxidative stress after overexpression of PSMB5

The decreased viability of hBMSCs following environmental insult has been regarded as a hallmark of replicative senescence. We accordingly investigated whether retaining the normal proteasomal activity could decelerate the aging process and strengthen the resistance to oxidative stress. The SA-β-gal assay was applied to identify the aged hBMSCs by staining them blue. As displayed in Fig. 3A, enhanced proteasomal activity by PSMB5 overexpression significantly reduced the percentage of blue cells from 55 ± 3% to 40 ± 4% ($p < 0.01$ vs. GFP control). In addition to reducing this senescent phenotype, PSMB5 overexpression also rendered aged hBMSCs less susceptible to oxidative challenge. By means of the oxidant-sensitive fluorophore DCFH-DA, we first confirmed that PSMB5 overexpression largely decreased the intracellular level of reactive oxygen species (ROS) in late-stage hBMSCs ($p < 0.01$ vs. GFP control; Fig. 3B). Whereas the CCK-8 assay demonstrated that H_2O_2 treatment lowered viability in a concentration-dependent manner (Suppl. Fig. 2), PSMB5-overexpressing late-stage hBMSCs exhibited better survival to oxidative insult. PSMB5 overexpression increased 450 nm absorbance by 40%, in comparison to that of the GFP control after exposure to the same concentration of H_2O_2 (300 μM, $p < 0.01$; Fig. 3Ca). Proteasomal activity was concurrently restored ($p < 0.01$; Fig. 3Cb). Taken together, these data indicated that PSMB5 overexpression could reverse the impairment of proteasomal functions, making aged cells less vulnerable to stressors and consequently rejuvenated.

3.4. Restored stemness of late-stage hBMSCs following PSMB5 overexpression

Human BMSCs are capable of differentiation into morphological and functional neuron-like cells [3]. Here we found that PSMB5 overexpression helped maintain their pluripotency. Consistent with prior reports, hBMSCs with multiple passages had a poorer differentiation ability as indicated by the lower percentage of Tuj1-positive ($Tuj1^+$) cells (22 ± 2%; Fig. 4A). Following the same one-week *in vitro* induction, PSMB5 overexpression raised this percentage in late-stage hBMSCs ($p > 0.05$ vs. Early-stage control; Fig. 4A), the effect of which was similar to that induced by the proteasome activator 18α-GA [12]. This result underscored the predominance of PSMB5 to the overall proteasomal activity, and suggested that modulation of its expression may be a feasible strategy to counteract replicative senescence.

Next, we investigated whether PSMB5 overexpression was beneficial for aged hBMSCs to differentiate *in vivo*. Four weeks after brain transplantation of pre-differentiated cells [3], the percentage of viable $Tuj1^+$ cells in the PSMB5 overexpression group (i.e. double-positive for Flag and Tuj1; Fig. 4B and Cf) remained higher than that in the GFP control group *in vivo* (i.e. double-positive for GFP and Tuj1, $p < 0.05$; Fig. 4B and Cc). Remarkably, some of cells following PSMB overexpression appeared to extend neurite branches (arrows; Fig. 4Cf), resembling that of mature neurons, in contrast to their GFP counterparts which were still showing the undifferentiated round appearance (arrows;

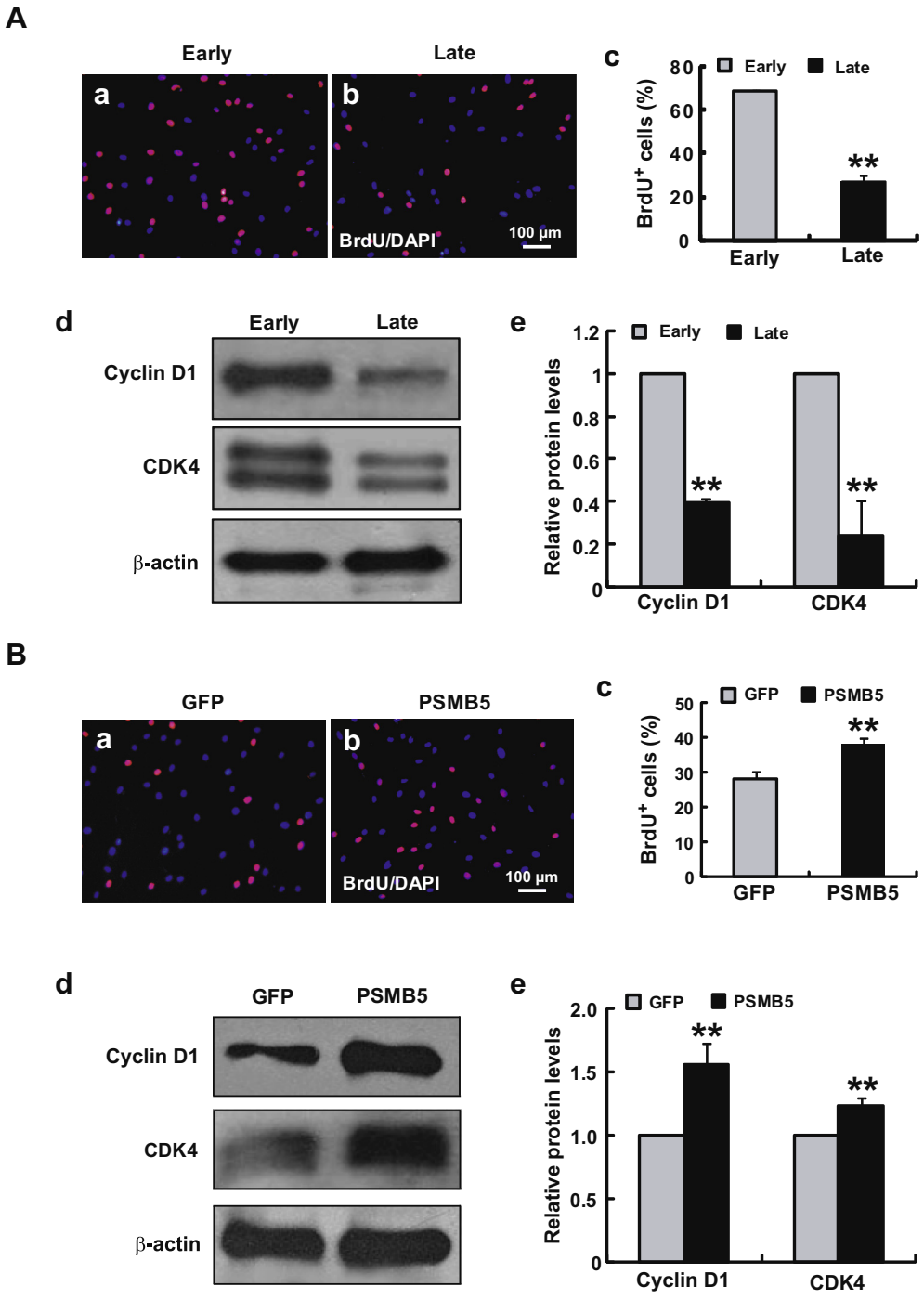

Fig. 2. Accelerated cell proliferation with upregulation of Cyclin D1/CDK4 in late-stage hBMSCs after PSMB5 overexpression. (A) Reduced cell proliferation accompanied with downregulation of Cyclin D1/CDK4 in late-stage hBMSCs. Comparison of BrdU-positive ($BrdU^+$) cells (red) in the early-stage (a) and the late-stage hBMSCs (b). (c) The quantitative data for $BrdU^+$ cells of (a) and (b). The cell cycle-related proteins Cyclin D1 and CDK4 were down-regulated in late-stage hBMSCs (d). (e) The quantitative data of (d). (B) Cell proliferation and Cyclin D1/CDK4 level were restored by PSMB5 overexpression. Comparison of $BrdU^+$ cells (red) in the GFP control (a) and the PSMB5-overexpressed late-stage hBMSCs (b). (c) The quantitative data for $BrdU^+$ cells of (a) and (b). PSMB5 overexpression attenuated the reduction of Cyclin D1/CDK4 in the late-stage hBMSCs (d). (e) The quantitative data of (d). All the experiments were performed in triplicate. $^{**}p < 0.01$ vs. Early-stage cells or GFP control. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 4Cc). This phenomenon suggested an enhanced neuronal differentiation of aged hBMSCs by PSMB5 overexpression. Altogether, the data demonstrated that maintenance of normal proteasomal activity by PSMB5 overexpression in aged hBMSCs preserves their potential for neuronal differentiation both *in vitro* and *in vivo*, thereby making such cells applicable to clinical transplantation even after prolonged culture expansion.

4. Discussion

The proteasome acts as a critical regulator of cellular homeostasis through its degradation of unwanted proteins. Maintaining normal proteolytic activity has been considered indispensable for self-renewal and integrity of stem cells [22,23]. In the present study, we explored the potential restoration of proteasomal

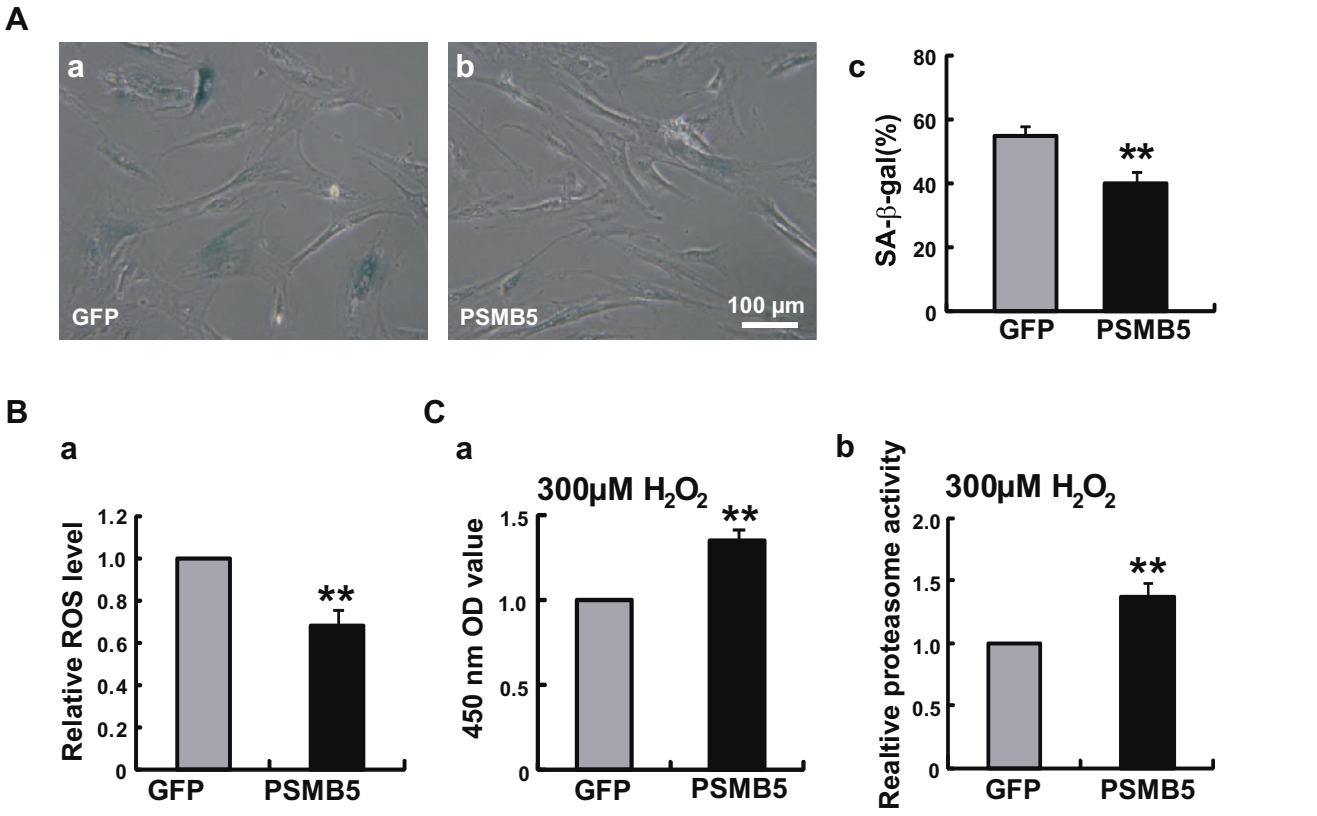

Fig. 3. Reduced appearance of senescent phenotypes by PSMB5 overexpression in the late-stage hBMSCs. (A) The SA-β-gal staining was applied to identify the aged cells (blue) in the late-stage hBMSCs with GFP (a) or PSMB5 (b) overexpression. (c) The quantitative data of (a) and (b). (B) Intracellular ROS levels were quantified by the DCF fluorescence. (C) The CCK-8 assay indicated that PSMB5 overexpression promoted cell survival following a 30-min oxidative challenging with H_2O_2 (300 μM) (a), concurrent with an increased proteasomal activity (b). Each experiment was repeated three times. $^{**}p < 0.01$ vs. GFP control. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

dysfunction in the aged hBMSCs. Our results showed that, although 20S proteasomal activity concurrent with self-renewing capacity progressively decreased after long-term *in vitro* passages, rectifying proteolytic abnormality by overexpressing the rate-limiting catalytic subunit PSMB5 helped preserve the stemness of hBMSCs, as well as rejuvenate them as evidenced by the enhanced resistance to oxidative stress.

In order to obtain a sufficient number of cells for clinical applications, hBMSCs have to undergo *in vitro* expansion which inevitably leads to cell aging. Replicative senescence in hBMSCs is a complicated process involving multiple alternations in morphological and functional phenotypes [24–26], presenting a challenge in trying to develop approaches to slow down or reverse senescent progression. Multiple factors have been suggested to contribute to this procedure, including shortened telomere length [27], reduced telomerase activity [28], oxidative stress insults [9,29], interruption of certain signaling pathways such as ERK [30] and TGF-β [31], as well as compromised proteasomal degradation [9,12,29,31]. Efforts are underway to minimize these pathogenic factors. Nevertheless, not all of these methods are clinically applicable. As an example, although simian virus 40 large T (SV40T) antigen could promote DNA replication and cell proliferation [32], its overexpression potentially results in a rising incidence of cancer [33].

Unlike some targets which are difficult to manipulate safely, the proteasome seems a better option for the purpose of counteracting replicative senescence due to several considerations. First, the proteasomal system is an innate component of normal mammalian cells and acts exclusively on disposal of unhealthy or excessive proteins [6]. So far, there has been little information concerning serious side-effects even with its hyper-activation. Second, proteasomal activity has been confirmed to be closely related to the preservation of hBMSC pluripotency [12]. Upon restoration of its normal function, the aged cells could be rejuvenated possibly through reconstruction of cellular homeostasis; this is of particular importance when the primarily-isolated cells have already entered the aging process, because it may possibly increase the population of qualified clinical donors. Third, the promotion of proteasomal activity could be readily attained by raising the expression of certain dominant catalytic subunits, as indicated here. Our results suggest at least two ways for achieving this goal, applying proteasomal activators such as 18α-GA that has been extensively used in clinics [12], or manipulating PSMB5 expression, which can maintain proteasomal activity longer after transplantation. Notably, the benefits of retaining the normal proteasomal function are multi-faceted. The present data substantiated that PSMB5 overexpression-restored proteolytic ability in the aged hBMSCs not only accelerated proliferation, reduced senescence and increased resistance to oxidative stress, but also recovered stem cell capabilities, as supported by the enhanced neuronal differentiation both *in vitro* and *in vivo*. Interestingly, we observed that late-stage hBMSCs with GFP remained rounded one month after transplantation, whereas those with PSMB5 overexpression were better differentiated; some of the latter extended neuron-like long branches from cell somas. In addition, several publications have demonstrated potential cross-talk between the proteasome and other cascades hypothesized to regulate replicative senescence. Modulation of certain signal transduction, such as insulin growth-like factor pathway, may activate proteasomal activity to offset oxidative stress insults and reduce age-related changes in the brain [34]. The

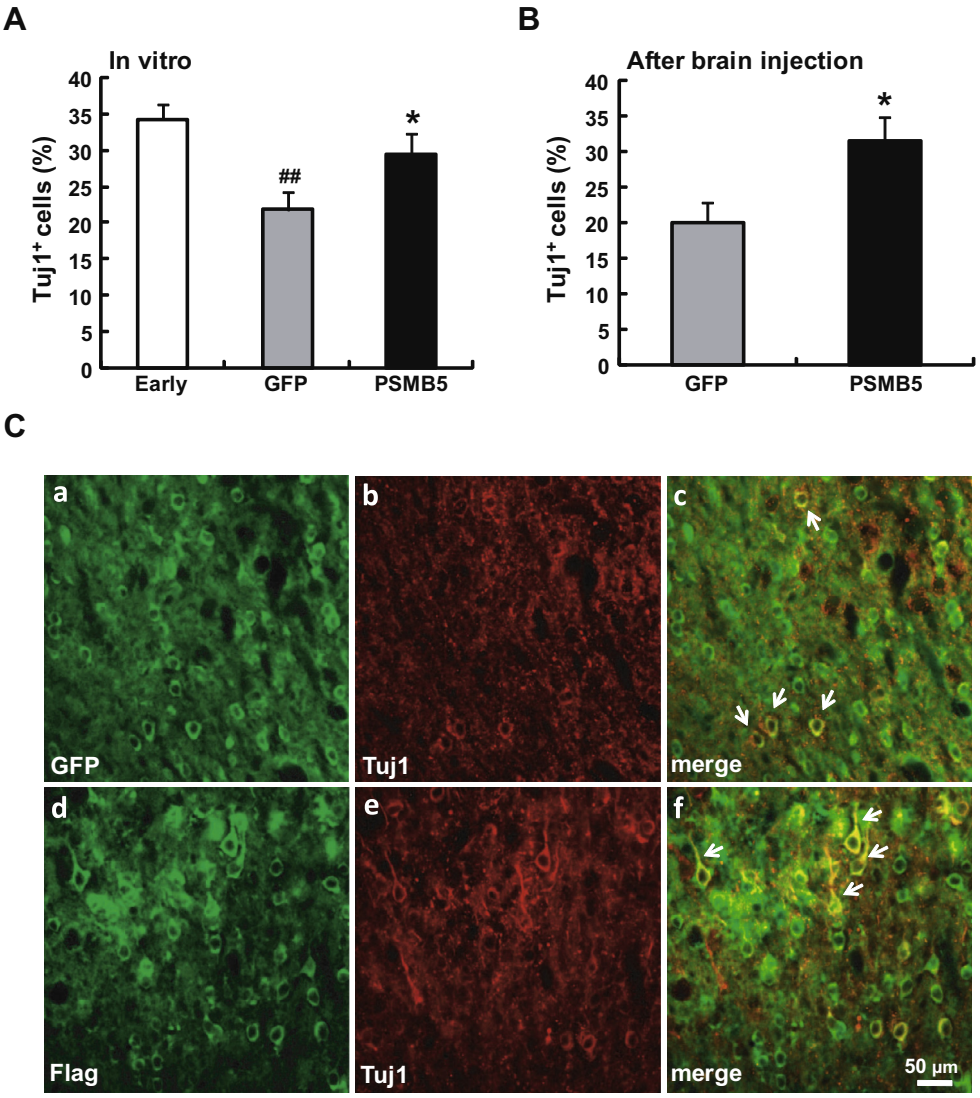

Fig. 4. Promoted pluripotency of late-stage hBMSCs after PSMB5 overexpression. (A) Immunophenotypic analysis by the neuronal marker Tuj1 staining showed that PSMB5 overexpression could attenuate the impairment of neuronal differentiation *in vitro*. Data were obtained from three independent experiments. $^{\#\#}p < 0.01$ vs. Early-stage cells; $^{*}p < 0.05$ vs. GFP control. (B) Four weeks after transplantation, pre-differentiated late-stage hBMSCs with PSMB5 overexpression still remained a better neuronal differentiation *in vivo*. $^{*}p < 0.05$ vs. GFP control. (C) Brain sections after transplantation were co-stained by antibodies against reporter genes [GFP (a) and Flag (d)] and Tuj1 (b, e). PSMB5-overexpressed cells showed preferable differentiation, as evidenced by their neuron-like branches (arrows; f), in contrast to the control counterparts which remained rounded morphology (arrows; c).

ubiquitin-proteasome pathway is also critical for telomerase activity [35], and telomere length and stability [36,37]. Moreover, the proteasome interacts with signaling pathways such as ERK [38] and TGF-β [39]. Hence, the functional proteasome is expected to provide a multi-faceted protection. Taken together, modulation of proteasomal activity seems safe, effective and feasible, and could therefore provide a superior strategy in dealing with replicative senescence of hBMSCs.

In summary, dysfunction of 20S proteasomes with reduced proteolytic ability potentially leads to replicative senescence in cultured hBMSCs, subsequently disqualifying them from clinical use. By overexpressing the rate-limiting PSMB5 which is progressively down-regulated after multiple passages, proteasomal activity is essentially restored, concurrent with suppressed senescent phenotypes as evidenced by (1) accelerated cell propagation through upregulation of Cyclin D1 and CDK4, (2) enhanced neuronal differentiation both *in vivo* and *in vitro*, and (3) improved cell vigor and resistance to oxidative stress. Out study examined the feasibility of maintaining stem cell integrity by counteracting compromised proteasomal activity with PSMB5 overexpression, which provides an entry point for large-scale production of hBMSCs without replicative senescence.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.12.113.

References

[1] J. Lee, J.H. Baek, K.S. Choi, H.S. Kim, H.Y. Park, G.H. Ha, H. Park, K.W. Lee, C.G. Lee, D.Y. Yang, H.E. Moon, S.H. Paek, C.W. Lee, Cyclin-dependent kinase 4 signaling acts as a molecular switch between syngenic differentiation and neural transdifferentiation in human mesenchymal stem cells, Cell Cycle 12 (2013) 442–451.

[2] V.B. Fernandez Vallone, M.A. Romaniuk, H. Choi, V. Labovsky, J. Otaegui, N.A. Chasseing, Mesenchymal stem cells and their use in therapy: what has been achieved?, Differentiation 85 (2013) 1–10

[3] L. Lu, X. Chen, C.W. Zhang, W.L. Yang, Y.J. Wu, L. Sun, L.M. Bai, X.S. Gu, S. Ahmed, G.S. Dawe, Z.C. Xiao, Morphological and functional characterization of predifferentiation of myelinating glia-like cells from human bone marrow stromal cells through activation of F3/Notch signaling in mouse retina, Stem Cells 26 (2008) 580–590.

[4] X. Zeng, M.S. Rao, Human embryonic stem cells: long term stability, absence of senescence and a potential cell source for neural replacement, Neuroscience 145 (2007) 1348–1358.

[5] B. Gharibi, F.J. Hughes, Effects of medium supplements on proliferation, differentiation potential, and in vitro expansion of mesenchymal stem cells, Stem Cells Transl. Med. 1 (2012) 771–782.

[6] N. Chondrogianni, E.S. Gonos, Proteasome dysfunction in mammalian aging: steps and factors involved, Exp. Gerontol. 40 (2005) 931–938.

[7] D. Li, H. Li, T. Wang, H. Pan, G. Lin, H. Li, Structural basis for the assembly and gate closure mechanisms of the *Mycobacterium tuberculosis* 20S proteasome, EMBO J. 29 (2010) 2037–2047.

[8] D. Voges, P. Zwickl, W. Baumeister, The 26S proteasome: a molecular machine designed for controlled proteolysis, Annu. Rev. Biochem. 68 (1999) 1015–1068.

[9] N. Chondrogianni, F.L. Stratford, I.P. Trougakos, B. Friguet, A.J. Rivett, E.S. Gonos, Central role of the proteasome in senescence and survival of human fibroblasts: induction of a senescence-like phenotype upon its inhibition and resistance to stress upon its activation, J. Biol. Chem. 278 (2003) 28026–28037.

[10] N. Chondrogianni, C. Tzavelas, A.J. Pemberton, I.P. Nezis, A.J. Rivett, E.S. Gonos, Overexpression of proteasome beta5 assembled subunit increases the amount of proteasome and confers ameliorated response to oxidative stress and higher survival rates, J. Biol. Chem. 280 (2005) 11840–11850.

[11] U. Ponnappan, M. Zhong, G.U. Trebilcock, Decreased proteasome-mediated degradation in T cells from the elderly: a role in immune senescence, Cell. Immunol. 192 (1999) 167–174.

[12] L. Lu, H.F. Song, W.G. Zhang, X.Q. Liu, Q. Zhu, X.L. Cheng, G.J. Yang, A. Li, Z.C. Xiao, Potential role of 20S proteasome in maintaining stem cell integrity of human bone marrow stromal cells in prolonged culture expansion, Biochem. Biophys. Res. Commun. 422 (2012) 121–127.

[13] S. Yamanaka, Elite and stochastic models for induced pluripotent stem cell generation, Nature 460 (2009) 49–52.

[14] N. Chondrogianni, E.S. Gonos, Proteasome activation as a novel antiaging strategy, IUBMB Life 60 (2008) 651–655.

[15] N. Chondrogianni, I. Petropoulos, C. Franceschi, B. Friguet, E.S. Gonos, Fibroblast cultures from healthy centenarians have an active proteasome, Exp. Gerontol. 35 (2000) 721–728.

[16] Y. Hirano, T. Kaneko, K. Okamoto, M. Bai, H. Yashiroda, K. Furuyama, K. Kato, K. Tanaka, S. Murata, Dissecting beta-ring assembly pathway of the mammalian 20S proteasome, EMBO J. 27 (2008) 2204–2213.

[17] W. Yang, A.Y. Lau, S. Luo, Q. Zhu, L. Lu, Characterization of amyloid-beta precursor protein intracellular domain-associated transcriptional complexes in SH-SY5Y neurocytes, Neurosci. Bull. 28 (2012) 259–270.

[18] W. Yang, X. Guo, S. Thein, F. Xu, S. Sugii, P.W. Baas, G.K. Radda, W. Han, Regulation of adipogenesis by cytoskeleton remodelling is facilitated by acetyltransferase MEC-17-dependent acetylation of alpha-tubulin, Biochem. J. 449 (2013) 605–612.

[19] G. Carrard, A.L. Bulteau, I. Petropoulos, B. Friguet, Impairment of proteasome structure and function in aging, Int. J. Biochem. Cell Biol. 34 (2002) 1461–1474.

[20] B. Dahlmann, Role of proteasomes in disease, BMC Biochem. 8 (Suppl. 1) (2007) S3.

[21] S.A. Datar, M. Galloni, A. de la Cruz, M. Marti, B.A. Edgar, C. Frei, Mammalian cyclin D1/Cdk4 complexes induce cell growth in Drosophila, Cell Cycle 5 (2006) 647–652.

[22] D. Vilchez, L. Boyer, I. Morantte, M. Lutz, C. Merkwirth, D. Joyce, B. Spencer, L. Page, E. Masliah, W.T. Berggren, F.H. Gage, A. Dillin, Increased proteasome activity in human embryonic stem cells is regulated by PSMD11, Nature 489 (2012) 304–308.

[23] S.P. Atkinson, J. Collin, N. Irina, G. Anyfantis, B.K. Kyung, M. Lako, L. Armstrong, A putative role for the immunoproteasome in the maintenance of pluripotency in human embryonic stem cells, Stem Cells 30 (2012) 1373–1384.

[24] I. Russo, A.R. Silver, A.P. Cuthbert, D.K. Griffin, D.A. Trott, R.F. Newbold, A telomere-independent senescence mechanism is the sole barrier to Syrian hamster cell immortalization, Oncogene 17 (1998) 3417–3426.

[25] S. Gagos, D. Iliopoulos, S. Tseleni-Balafouta, M. Agapitos, C. Antachopoulos, A. Kostakis, P. Karayannakos, G. Skalkeas, Cell senescence and a mechanism of clonal evolution leading to continuous cell proliferation, loss of heterozygosity, and tumor heterogeneity: studies on two immortal colon cancer cell lines, Cancer Genet. Cytogenet. 90 (1996) 157–165.

[26] J.C. Angello, W.R. Pendergrass, T.H. Norwood, J. Prothero, Cell enlargement: one possible mechanism underlying cellular senescence, J. Cell. Physiol. 140 (1989) 288–294.

[27] J.F. Riou, L. Guittat, P. Mailliet, A. Laoui, E. Renou, O. Petitgenet, F. Megnin-Chanet, C. Helene, J.L. Mergny, Cell senescence and telomere shortening induced by a new series of specific G-quadruplex DNA ligands, Proc. Natl. Acad. Sci. USA 99 (2002) 2672–2677.

[28] C. Pan, B.H. Xue, T.M. Ellis, D.J. Peace, M.O. Diaz, Changes in telomerase activity and telomere length during human T lymphocyte senescence, Exp. Cell Res. 231 (1997) 346–353.

[29] H. Unterluggauer, B. Hampel, W. Zwerschke, P. Jansen-Durr, Senescence-associated cell death of human endothelial cells: the role of oxidative stress, Exp. Gerontol. 38 (2003) 1149–1160.

[30] A. Lorenzini, M. Tresini, M. Mawal-Dewan, L. Frisoni, H. Zhang, R.G. Allen, C. Sell, V.J. Cristofalo, Role of the Raf/MEK/ERK and the PI3K/Akt(PKB) pathways in fibroblast senescence, Exp. Gerontol. 37 (2002) 1149–1156.

[31] R. Cipriano, C.E. Kan, J. Graham, D. Danielpour, M. Stampfer, M.W. Jackson, TGF-beta signaling engages an ATM-CHK2-p53-independent RAS-induced senescence and prevents malignant transformation in human mammary epithelial cells, Proc. Natl. Acad. Sci. USA 108 (2011) 8668–8673.

[32] M. Gong, Y. Bi, W. Jiang, Y. Zhang, L. Chen, N. Hou, Y. Liu, X. Wei, J. Chen, T. Li, Immortalized mesenchymal stem cells: an alternative to primary mesenchymal stem cells in neuronal differentiation and neuroregeneration associated studies, J. Biomed. Sci. 18 (2011) 87.

[33] J.R. Pietruska, A.B. Kane, SV40 oncoproteins enhance asbestos-induced DNA double-strand breaks and abrogate senescence in murine mesothelial cells, Cancer Res. 67 (2007) 3637–3645.

[34] E. Crowe, C. Sell, J.D. Thomas, G.J. Johannes, C. Torres, Activation of proteasome by insulin-like growth factor-I may enhance clearance of oxidized proteins in the brain, Mech. Ageing Dev. 130 (2009) 793–800.

[35] W. Zhang, Q. Tong, S. Li, X. Wang, Q. Wang, MG-132 inhibits telomerase activity, induces apoptosis and G(1) arrest associated with upregulated p27kip1 expression and downregulated survivin expression in gastric carcinoma cells, Cancer Invest. 26 (2008) 1032–1036.

[36] M. McKerlie, S. Lin, X.D. Zhu, ATM regulates proteasome-dependent subnuclear localization of TRF1, which is important for telomere maintenance, Nucleic Acids Res. 40 (2012) 3975–3989.

[37] Y.R. Her, I.K. Chung, Ubiquitin ligase RLIM modulates telomere length homeostasis through a proteolysis of TRF1, J. Biol. Chem. 284 (2009) 8557–8566.

[38] X. Deschenes-Simard, M.F. Gaumont-Leclerc, V. Bourdeau, F. Lessard, O. Moiseeva, V. Forest, S. Igelmann, F.A. Mallette, M.K. Saba-El-Leil, S. Meloche, F. Saad, A.M. Mes-Masson, G. Ferbeyre, Tumor suppressor activity of the ERK/MAPK pathway by promoting selective protein degradation, Genes Dev. 27 (2013) 900–915.

[39] T. Sezaki, L. Tomiyama, Y. Kimura, K. Ueda, N. Kioka, Dlg5 interacts with the TGF-beta receptor and promotes its degradation, FEBS Lett. 587 (2013) 1624-1629.