Prion protein is expressed on long-term repopulating hematopoietic stem cells and is important for their self-renewal

Cheng Cheng Zhang*, Andrew D. Steele**, Susan Lindquist**,†, and Harvey F. Lodish**

*Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142; and †Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02142

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Although the wild-type prion protein (PrP) is abundant and widely expressed in various types of tissues and cells, its physiological function(s) remain unknown, and PrP knockout mice do not exhibit overt and undisputed phenotypes. Here we showed that PrP is expressed on the surface of several bone marrow cell populations successively enriched in long-term (LT) hematopoietic stem cells (HSCs) using flow cytometry analysis. Affinity purification of the PrP-positive and -negative fractions from these populations, followed by competitive bone marrow reconstitution assays, shows that all LT HSCs express PrP. HSCs from PrP-null bone marrow exhibited impaired self-renewal in serial transplantation of lethally irradiated mouse recipients both in the presence and absence of competitors. When treated with a cell cycle-specific myelotoxic agent, the animals reconstituted with PrP-null HSCs exhibited increased sensitivity to hematopoietic cell depletion. Ectopic expression of PrP in PrP-null bone marrow cells by retroviral infection rescued the defective hematopoietic engrafment during serial transplantation. Therefore, PrP is a marker for HSCs and supports their self-renewal.

Bone marrow | serial transplantation | hematopoiesis | reconstitution

The difficulty in purification and study of hematopoietic stem cells (HSCs) is hampered by our limited understanding of the proteins that are specifically expressed on their surface (1–3). Furthermore, few HSC markers are of functional significance for HSC activity (4). We characterized a population of mouse fetal liver cells that express the CD34 antigen (CD34+ and support the ex vivo expansion of HSCs, and showed that they express abundant amounts of prion protein (PrP) (5). PrP is a highly conserved glycoprotein tethered to cell membranes by a glycosylphosphatidylinositol (GPI) anchor that is expressed on hematopoietic cells as well as in many tissues including brain, heart, and muscle (6–8). Although it is well established that PrP is the primary component of infectivity in prion diseases (9), its normal function(s) remains obscure. Several roles for PrP have been suggested, including copper uptake, cell signaling, cell survival, protection against oxidative stress, cell adhesion, and differentiation (10, 11). However, PrP-null mice exhibit no consistent, overt, phenotype other than resistance to infection with prions (12, 13). Here we demonstrate that PrP is a surface marker for HSCs and is required for their self-renewal, as judged by successive bone marrow transplantations.

Results

PrP Is a Marker for Long-Term (LT) HSCs. Preliminary studies showed that 40% of adult mouse bone marrow (BM) cells express PrP on their surface. More than 80% of these PrP+ cells were erythroid cells as they expressed the glycophorin-related surface protein Ter119 (Ter119+) (data not shown). In Fig. 1 the expression of the PrP protein was monitored on the surface of WT mouse BM cell populations that were progressively enriched for HSCs. One way to enrich HSCs is isolation of the side population (SP) fraction of adult BM cells, which is identified by low accumulation of Hoechst dye.

Conflict of interest statement: No conflicts declared.

Abbreviations: HSC, hematopoietic stem cell; PrP, prion protein; GPI, glycosylphosphatidylinositol; BM, bone marrow; SP, side population; LT, long term; 5-FU, 5-fluoracil.

*To whom correspondence may be addressed. E-mail: lodish@wi.mit.edu or lindquist.admin@wi.mit.edu.

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Lin^−/H11001^−/H11002^−/H11003^−/Sca-1^−^ BM cells were sorted according to their cell surface expression of PrP. PrP^+^ and PrP^−^ fractions were mixed separately with \(1 \times 10^5\) CD45.1 total BM cells and injected into lethally irradiated CD45.1 recipients. A total of \(1 \times 10^5\) PrP^+^ BM cells and PrP^−^ cells supported both ST and LT HSC activity, as assessed by reconstitution of peripheral blood cells (Fig. 2A, bars 2 and 4). PrP^+^ donor cells repopulated both lymphoid and myeloid compartments (Fig. 2B).

Next, HSC-enriched Lin^−/Sca-1^+^ BM cells were sorted according to their expression of PrP. Of Lin^−^ cells, 0.8% and 5.2% are Sca-1^−^PrP^+^ and Sca-1^−^PrP^−^, respectively (data not shown). Competitive transplantation of 500 isolated Lin^−/Sca-1^−^PrP^+^ cells resulted in significant repopulation after 3 weeks (Fig. 2C, bar 1) but none after 4 months (bar 3). In contrast, injection of only 100 Lin^−^Sca-1^−^PrP^+^ CD45.2 donor BM cells resulted in significant repopulation after both 3 weeks and 4 months (bars 2 and 4, respectively). Each of the five recipient mice was engrafted with frequencies of 5.1%, 1.4%, 27.6%, 2.5%, and 1.4% of peripheral blood cells. Thus, starting with a purified Lin^−/Sca-1^−^ HSC population, the subtraction of cells that express PrP on their surface contain all LT HSCs.

Fig. 2D further shows that all LT HSCs express PrP on their surface. SP cells were sorted based on PrP expression. SP cells that did not express PrP contained no LT HSC activity, whereas those that expressed PrP had significant activity (Fig. 2D). Thus, adult BM LT HSC activity resides in the PrP^+^ but not in the PrP^−^ fractions of both Lin^−^Sca-1^+^ and SP populations. In the transplantation experiments in Fig. 2A and C, we used 5-fold more PrP^−^ cells than PrP^+^ cells. From the data in Fig. 2A, we conclude that the presence of surface PrP protein distinguishes those Lin^−/Sca-1^−^ cells that contain the LT HSCs from those that do not. The ability of PrP^+^ SP cells to repopulate all recipients coupled with no detectable LT HSC activity in the PrP^+^-SP population (Fig. 2D) similarly suggests that all LT HSCs express PrP. We conclude that PrP is a marker for LT HSCs. Like other LT HSC markers, PrP expression is not exclusive to that cell type.

**PrP Is Important for Renewal of HSCs Under Stress.** We asked whether PrP expression had a functional role in HSCs by using mice ablated for PrP. The PrP-null and WT control mice used in the first studies (Figs. 3 and 4) were backcrossed onto the C57BL/6J background four times (termed N4). First, we analyzed the nature and number of hematopoietic cells from WT and PrP-null littersmates (18). The hematocrits, hemoglobin levels, and total red and white blood cell levels in peripheral blood were similar in the PrP-null and control mice (Table 2, which is published as supporting information on the PNAS web site). Furthermore, BM cells were reacted with various lineage-specific antibodies, and the cells were analyzed by flow cytometry; we found no differences in staining profiles between the two types of animals (data not shown). Consistent with prior observations (19), we assayed PrP-null and WT BM cells for colony-forming units (CFUs) to look for defects in progenitor numbers or activities. PrP-null and control BM had similar numbers of multipotential granulocyte/erythroid/monocyte/megakaryocyte progenitors (CFU-GEMM), granulocyte/monocyte progenitors (CFU-GM), erythroid progenitors (BFU-E, burst-forming unit), and B lymphoid (CFU-Pre-B) progenitors (Fig. 3A). In addition, the surface expression of seven important hematopoietic surface antigens on BM SP cells isolated from PrP-null and WT mice was similar, as detected by flow cytometry analysis (Table 3, which is published as supporting information on the PNAS web site). Thus, PrP-deficient BM has normal levels of progenitors and terminally differentiated hematopoietic cells.
To determine whether PrP has a role in activity of HSCs, we used competitive reconstitution assays (20). Fig. 3B shows the competitive repopulation results pooled from three independent experiments. A total of 2 × 10^6 PrP-null or WT CD45.2 BM cells were mixed with an equal number of competitor CD45.1 BM cells and transplanted into lethally irradiated CD45.1 recipients. If the PrP-null or control WT donor CD45.2 BM cells functioned equivalently to the CD45.1 competitor, one would expect 50% of the peripheral blood cells in the recipient to express the CD45.2 protein. Indeed, both WT and PrP-null donor cells exhibited ~50% reconstitution of peripheral blood 4 months after transplant (Fig. 3B, bars 1 and 2). This finding suggests that freshly isolated BM from PrP-null and WT mice have similar HSC numbers and activities. We tested the serial engraftment capacity of PrP-null HSCs by pooling BM cells from primary transplanted recipients and transplanting them into lethally irradiated secondary CD45.1 recipients. After 4 months, peripheral blood of the secondary recipients was analyzed for expression of the donor CD45.2 marker (Fig. 3B, bars 3 and 4). Cells derived from original WT CD45.2 BM comprised 60 ± 4% of the nucleated peripheral blood cells. In contrast, cells derived from the original PrP-null CD45.2 BM comprised only 29 ± 7% of the peripheral blood, a significantly lower number (P < 0.005, t test) than observed with WT donors. The lineage profiles of the primary and secondary transplanted PrP-null BM were normal (data not shown). Tertiary transplantations were carried out in the same manner. Again cells derived from the original PrP-null BM showed significantly less engraftment than their WT counterparts (Fig. 3B, bars 5–6, P < 0.05, t test). Because the contribution of PrP-null HSCs to the reconstitution of lethally irradiated recipients steadily decreased with each transplantation, relative to WT HSCs, we infer that PrP-null HSCs have impaired self-renewal capabilities as analyzed by LT serial transplantation.

Because the competitor CD45.1 cells used in the transplants in Fig. 3B may have had untoward effects on the CD45.2 WT or PrP-null cells, we carried out serial transplantation without competitors. To this end 1 × 10^6 BM cells isolated from three PrP-null or WT littermates were transplanted into lethally irradiated CD45.1 recipients. As expected all of the recipients survived (Fig. 3C, bars 1 and 2). Four months later, 5 × 10^5 BM cells from these primary recipients were pooled and transplanted into secondary recipients, and the same procedure was repeated in tertiary transplantations. After the secondary transplant, 86% of the mice receiving WT BM survived, whereas only 57% of those receiving PrP-null BM did (Fig. 3C, bars 3 and 4). A more striking difference in survival between PrP-null and WT repopulated mice was noted after tertiary transplantation: 67% of transplanted mice receiving WT BM survived, whereas none of the mice transplanted with null BM died (Fig. 3C, bars 5 and 6). In all cases, flow cytometry analysis of the transplanted mice confirmed that the recipient BM derived exclusively from the donor cells (data not shown). The defect in the ability of PrP-null BM to repopulate during serial transplantation was confirmed in a separate experiment in which a higher number of cells (1 × 10^6 cells) from the secondary transplant were used to transplant lethally irradiated tertiary recipients. The survival curve confirmed the dramatically lower repopulation potential of PrP-null BM after the tertiary transplantation (Fig. 3D, P < 0.0001, log-rank test).

Because the PrP-null mice used in these studies were only backcrossed four times into the C57BL/6J background, it remained possible that some genetic difference linked to the PrP locus was responsible for the observed differences in serial transplantation. Thus, in the experiment depicted in bars 7–9 of Fig. 3C, we used a retroviral vector to introduce PrP into the PrP-null BM cells isolated from the secondary transplanted recipients. The efficiency of infection was 30–50% (Fig. 5, which is published as supporting information on the PNAS web site); specifically, 1 × 10^5 cells were infected and transplanted into lethally irradiated recipients. Consistent with the data in bar 5, none of the mice transplanted with BM

Fig. 2.  All LT repopulating bone marrow HSC cells express surface PrP. A total of 1 × 10^6 PrP− or 2 × 10^6 PrP+ CD45.2 donor BM cells (A), 2 × 10^6 PrP− CD45.2 donor BM cells (B), 500 sorted Lin− Sca−1+ PrP− or 100 Lin− Sca−1+ PrP+ CD45.2 donor BM cells (C), or 500 isolated SP PrP− or 250 SP PrP+ donor BM cells (D) were mixed with 1 × 10^5 competitor CD45.1 cells and transplanted into lethally irradiated CD45.1 mice (n = 4–5). (A) Donor CD45.2 contribution at 4 weeks and 6 months after transplant. (B) Multilineage contribution at 6 months after transplant. (C) Donor contribution at 3 weeks and 4 months after transplant. (D) Donor contribution at 4 months after transplant.
infected by the control GFP vector survived (Fig. 3C, bar 7). In contrast, half of the mice transplanted with the same BM but expressing exogenous PrP survived (Fig. 3C, bar 8 and Fig. 6, which is published as supporting information on the PNAS web site, \( P < 0.05 \), log-rank test). In another control experiment, the same BM cells were infected with a retrovirus encoding a mutant PrP with deletion of amino acids 23–72, the segment that contains the N-terminal octapeptide repeats (11). None of the mice transplanted with BM expressing this mutant PrP protein survived (Fig. 3C, bar 9, and Fig. 6). This deletion might also remove amino acids that may be essential for the internalization or the proper signal peptide processing (21, 22), and further study is needed to characterize the nature of PrP sequence critical for supporting PrP’s HSC engraftment. However, this complementation experiment demonstrates that full-length PrP supports hematopoietic engraftment during long-term transplantation. It will be interesting to further study whether the hematopoietic rescue is PrP dose dependent, and whether overexpression of PrP in WT bone marrow gains better serial engraftment capacity.

The competitive reconstitution experiment in Fig. 3E confirms our conclusion that the defects observed in the PrP-null BM during serial transplantations indeed are due to defects in HSC renewal. BM from the initially transplanted mice used in bars 1 and 2 of Fig. 3C was mixed with \( 5 \times 10^5 \) freshly isolated BM cells from WT CD45.1 mice and transplanted into irradiated WT CD45.1 recipients. A total of \( 2 \times 10^6 \) donor CD45.2 PrP-null or littermate control BM cells were mixed with \( 2 \times 10^5 \) CD45.1 WT BM cells and transplanted into lethally irradiated CD45.1 recipients (\( n = 6 \)). The extent of chimera in peripheral blood (bars 1 and 2) was analyzed 4 months after transplant. In the experiment in bars 3 and 4, BM cells from the primary transplanted mice were pooled, and \( 2 \times 10^5 \) cells were injected directly into each of five lethally irradiated CD45.1 recipients. The fraction of donor CD45.2 cells in the peripheral blood of these transplanted mice was analyzed 4 months later. The process was repeated for the tertiary transplants (bars 5 and 6). This is a combined result of three independent experiments from a total of initial six null or wild-type control mice. *, significantly different from bar 3 value, \( P < 0.005 \); **, significantly different from bar 5 value, \( P < 0.005 \). (C) Serial transplantation of PrP-null and WT BM cells without competitors; rescue of HSC activity in PrP-null cells by PrP expression. A total of \( 1 \times 10^5 \) PrP-null or WT BM CD45.2 cells, pooled from three donors, were transplanted into lethally irradiated CD45.1 recipients without competitors. Recipients were monitored daily for survival for >30 days (bars 1 and 2, \( n = 6 \)). These mice were killed after 4 months. From them, \( 5 \times 10^5 \) BM cells were collected and transplanted into new irradiated recipients (bars 3 and 4, \( n = 7 \)). The process was repeated an additional time for tertiary transplants (bars 5 and 6, \( n = 12 \)). In parallel, \( 1 \times 10^5 \) PrP-null BM cells isolated from the surviving secondary transplant recipients, as shown in bar 3, were infected by retroviruses encoding GFP, PrP, or PrP Δ23-72, and injected into irradiated recipients (bars 7–9, \( n = 7–8 \)). Plotted is the fraction of surviving mice 50 days after each bone marrow transplant. See Fig. 6 for details of animal survival. (D) A total of \( 1 \times 10^5 \) BM cells from the secondary transplant mice shown in bars 3 and 4 of C were transplanted into the lethally irradiated recipients. Survival data were plotted as Kaplan–Meier curves (\( n = 11 \) for each group, \( P < 0.0001 \), log-rank test). (E) Competitive transplantation demonstrates impaired renewal of PrP-null HSC activity during successive bone marrow transplants. Here, \( 5 \times 10^5 \) PrP-null or WT BM collected from primary transplanted mice 4 months after transplant (without competitors, as in C, bars 1 and 2) were mixed with \( 5 \times 10^5 \) CD45.1 freshly isolated BM cells and transplanted into lethally irradiated recipients. Peripheral blood engraftment at 6 weeks and 5 months after transplant is shown (\( n = 4 \)). *, significantly different from bar 1 value, \( P < 0.005 \); **, significantly different from bar 3 value, \( P < 0.05 \).
were collected and their HSC activities were measured by competitive reconstitution. PrP-null BM cells showed 2.2 ± 0.9% engraftment, significantly lower than that from WT cells, which had 5.9 ± 1.4% engraftment (Fig. 7D, bars 1 and 2, P < 0.05, t test). In parallel, we infected N6 PrP-null BM cells with either a PrP/GFP or control GFP retrovirus population, isolated the GFP-positive population, and then transplanted the cells without competitors into irradiated recipients. Three months later, the transplanted BM cells were collected and their HSC activities were measured in secondary transplants by competitive reconstitution (Fig. 7D, bars 3 and 4). Consistent with the results in bars 1 and 2, the expression of PrP increased the HSC activity of prion null BM (bar 3, 1.8 ± 0.4% engraftment by control GFP infected cells; bar 4, 4.8 ± 1.9% engraftment by PrP infected cells, P < 0.05, t test). We conclude that although the absence of PrP does not affect HSC activity in normal unstreressed mice, it is important for renewal of HSC activity that occurs under the stress of serial bone marrow transplantation.

To further test the notion that PrP deficiency leads to a defect in the stress response of hematopoietic cells, we treated mice with 5-fluorouracil (5-FU), which is toxic to cycling cells and accelerates the entry of HSCs into the cell cycle (23). Isogenic WT mice reconstituted with either PrP-null or control BM were treated with 5-FU at 1 month after transplantation. The survival of mice repopulated with PrP-null BM was significantly lower than those reconstituted with wild-type cells (Fig. 4A, P < 0.05, log-rank test). Repeated experiments showed similar results (data not shown).

We verified that PrP was still expressed on the surface of wild-type HSCs after 5-FU stress; in this experiment, BM cells from 5-FU-treated wild-type mice were sorted into PrP+ and PrP− fractions. Competitive repopulation assays showed that, as in normal BM, PrP+ cells contain all of the HSC activity (Fig. 4B). The 5-FU-treated mice used in the study in Fig. 4A were WT in all tissues except for their hematopoietic system, which was of either wild-type or PrP-null origin. Thus, the difference in survival after 5-FU treatment is due only to the presence or absence of the PrP on hematopoietic stem or early progenitor cells. Therefore, consistent with its role of supporting sustained LT HSC self-renewal in serial transplantation, PrP protects hematopoietic cells from exhaustion by toxic agents such as 5-FU.

Discussion

Previous work showed that several types of blood cells express PrP, albeit at vastly different levels: lymphocytes, dendritic cells, monocytes, granulocytes, erythrocytes, platelets, certain lymphoid precursors, and CD34+ cells, which in humans are an enriched stem/progenitor cell population (7, 24–27). However, it was unknown whether PrP is expressed on HSCs with repopulating activity, and there was no indication of a possible function of PrP in any of these cells. We demonstrated that PrP is located on the surface of HSCs and supports their engraftment during serial transplantation. Thus, using antibodies to PrP, together with other HSC markers, it may be possible to devise novel protocols for purifying human HSCs.

Other GPI-anchored proteins, including Sca-1 (20) and possibly CD59 (28) are also expressed on HSCs. Similar to PrP, Sca-1 is also required for HSC self-renewal (20). Like other GPI-anchored proteins (29), PrP has been reported to localize to lipid rafts in the plasma membrane (30), and it might regulate certain signaling proteins that are also concentrated in these domains. Indeed, PrP has been reported to be involved in activation of Fyn tyrosine kinase (31) and to interact with laminin, the laminin receptor, and stress-inducible protein 1, as well as other proteins (10). Like the GPI-anchored α-subunit of the ciliary neurotrophic factor receptor (32), PrP might be the coreceptor for a hormone affecting HSC activity, possibly concentrating this as yet unidentified molecule on the cell surface and/or presenting it to the signaling receptor(s). In this function, PrP might protect HSCs from apoptosis or sustain their long-term self-renewal. Alternatively, PrP might interact with proteins in the BM extracellular matrix or on the surface of stromal cells, and possibly support retention of transplanted HSCs within the BM microenvironment. Our work opens an avenue of investigation that may illuminate the details of the normal function of PrP in cell biology.

Materials and Methods

Mouse Strains and Genotyping. CD45.1 and CD45.2 C57BL/6 mice were purchased from The Jackson Laboratory or the National Cancer Institute. The PrP knockout mice (18) were provided by R. Race and B. Chesebro (Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, MT) and backcrossed to C57 BL/6 CD45.2 mice at least four or six times (indicated in text and figure legends) to obtain PrP-null and WT littermates. To genotype mice, DNA was extracted from proteinase K-digested tail tips by using a DNeasy kit according to the manufacturer's instructions (Qiagen, Valencia, CA). The PrP and/or neomycin (neo) insert was amplified in four-primer PCR using primers 5'-TCATCCCAAGATCAGGAAGTGA-G-3' and 5'-ATGGCGAACTTTGGCTACTGCTG-3' for PrP and 5'-TGTAGCCTCGGAGACAGTTTC-3' and 5’-GATGGATTG-CACGCAGTTTC-3' for the neomycin insert. The cycling conditions were 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 62°C for 30 s, and 72°C for 1 min, followed by a final extension of 72°C for 10 min.

Flow Cytometry. Donor BM cells were isolated from 7- to 10-week-old mice. Anti-PrP mAb (SAF-83; Cayman Chemical, Ann Arbor, MI) was FITC-conjugated by using the Quick-Tag FITC conjugation kit (Roche Diagnostics). Its specificity was verified by its inability to bind to PrP-null cells and its specific binding to cell lines expressing transfected prion protein (data not shown).

SP cells were stained with Hoechst dye 33342 as described (14). In Fig. 1A, Hoechst-stained cells were then costained with anti-PrP-FITC and phycoerythrin (PE)-conjugated anti-Sca-1, CD43, CD44, CD49D, CD49E, CD11A, or CD62L antibodies (BD Pharmingen). When Endoglin was detected, Hoechst 33342 stained cells were subsequently stained with anti-Endoglin mAb (BD Pharmingen), anti-rat-PE, and anti-PrP-FITC. In Fig. 1B, the cells were incubated for 1 h at 37°C with a 10% FCS/1×PBS solution, washed, and stained with anti-PrP-FITC and phycoerythrin (PE)-conjugated antibodies (BD Pharmingen) followed by Hoechst dye 33342 as described (14).
were stained first with anti-Endoglin mAb (BD Pharmingen) followed by anti-rat-PE/CY5.5 (eBioscience, San Diego, CA), a biotinylated antibody mixture recognizing multiple hematopoietic lineage markers (StemCell Technologies, Vancouver), as well as streptavidin-APC, anti-PrP-FTTC, and anti-Sca-1-PE (BD Pharmingen). In Fig. 2, cells were stained with anti-PrP-FTTC (Fig. 2A and B), or biotinylated multiligne antibody mixture followed by streptavidin-APC, anti-PrP-FTTC, and anti-Sca-1-PE (Fig. 2C). In Fig. 2D, the staining was the same as in Fig. 1A, plot 4.

The peripheral blood analysis after reconstitution was performed as described (5). Peripheral blood cells were stained with anti-CD45.2-FTTC and anti-CD45.1-PE, Anti-Thy1.2-PE, anti-B220-PE, anti-Mac-1-PE, anti-Gr-1-PE, and anti-Ter119-PE monoclonal antibodies (BD Pharmingen) were used for detecting specific hematopoietic lineages. FACS analyses were performed on a FACSCalibur instrument. Cells were sorted in a MoFlo cell sorter. The purity of sorted cells was typically >90%.

**Reconstitution Analysis.** The reconstitution protocol was essentially as described (5). Briefly, the indicated numbers of CD45.2 donor cells were injected directly, or after mixing with 1 × 10^6, 2 × 10^6, or 5 × 10^6 (as indicated) freshly isolated CD45.1 competitor BM cells, intravenously into a group of 6- to 9-week-old CD45.1 mice as described (5). Peripheral blood cells were stained with anti-streptavidin-APC, anti-PrP-FITC, and anti-Sca-1-PE (BD Pharmingen) for CFU-pre-B colonies, according to the manufacturer's protocols.

**Colony Assays.** PrP-null or WT BM cells were diluted to 2 × 10^5 per ml in Iscove's modified Dulbecco's medium (IMDM) with 2% FBS, and then were seeded into methylcellulose medium StemSpan (StemCell Technologies) in the presence of 10^4/ml SCF, 20 ng/ml IGF-2, and 10 ng/ml FGF-1 (33). Cells were then resuspended in viral supernatants (2 × 10^5 cells per ml) with 6 μg/ml polybrene and centrifuged at 720 × g for 90 min before culturing for 24 h in StemSpan (StemCell Technologies) in the presence of 10 μg/ml heparin, 10 ng/ml SCF, 20 ng/ml TPO, 20 ng/ml IGF-2, and 10 ng/ml FGF-1 (33). Colony size and cellularity were measured. The calculation of competitive repopulating units in limiting dilution experiments was conducted as described (5, 33), using L-CALC software (StemCell Technologies).

**Retrovirus Infection.** Mouse PrP (Δ23–72) was constructed by ligating the PCR products of PrPΔ1–23 and 72–254 and inserting into XZ201 (a MSCV-IRES-GFP vector, gift from Xiaowu Zhang, Whitehead Institute, Cambridge, MA). Similar PrP or PrP(Δ23–72) cDNA was cloned upstream of the internal ribosomal entry site (IRES) in XZ201. The retroviral plasmids were transfected by using lipofectamine 2000 (Invitrogen) into BOSC packaging cells. The resulting retroviral supernatant was collected 48 h later and was used for infection. To this end, BM cells were resuspended in viral supernatants (2 × 10^5 cells per ml) with 6 μg/ml polybrene and centrifuged at 720 × g for 90 min before culturing for 24 h in StemSpan (StemCell Technologies) in the presence of 10 μg/ml heparin, 10 ng/ml SCF, 20 ng/ml TPO, and 20 ng/ml IGF-2 and 10 ng/ml FGF-1 (33). Cells were then resuspended in viral supernatant for another round of infection. Cells were then used directly for transplantation or cultured for another 4 days before sorting.

**5-FU Challenge.** In Fig. 4A, 5 × 10^5 PrP-null or wild-type BM cells were used to reconstitute 10 Gy-irradiated CD45.1 C57BL/6 mice; there were 11 mice in each group. At 1 month after transplant, 5-FU was administered i.p. at a dose of 150 mg/kg weekly for 2 weeks. The survival rates of the two groups were analyzed by using a log-rank test (PRISM, GraphPad, San Diego). The survival rates of the two groups were analyzed by using a log-rank test (PRISM, GraphPad, San Diego).

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