ARTICLE - Hematopoiesis

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Cyp7b1-inhibiting azoles enhance hematopoietic stem and progenitor cell mobilization in normal and sickle cell disease mice

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Supplementary Methods

Mouse hematopoietic cell markers

The marker combinations used to identify mouse hematopoietic stem and progenitor cell populations examined in this study were:

HSC (hematopoietic stem cell): $CD150^{+}CD48^{-/low}Lineage^{-}Sca\text{-}1^{+}c\text{-}kit^{+}$

MPP (multipotent progenitor): $CD150^{-}CD48^{-/low}Lineage^{-}Sca\text{-}1^{+}c\text{-}kit^{+}$

HPC-1 (hematopoietic progenitor cell-1): $CD150^{-}CD48^{+}Lineage^{-}Sca\text{-}1^{+}c\text{-}kit^{+}$

HPC-2 (hematopoietic progenitor cell-2): $CD150^{+}CD48^{+}Lineage^{-}Sca\text{-}1^{+}c\text{-}kit^{+}$

CMP (common myeloid progenitor): $CD34^{+}CD16/32^{low}Lineage^{-}Sca\text{-}1^{-}c\text{-}kit^{+}$

GMP (granulocyte-macrophage progenitor): $CD34^{+}CD16/32^{high}Lineage^{-}Sca\text{-}1^{-}c\text{-}kit^{+}$

MEP (megakaryocyte-erythroid progenitor): $CD34^{-/low}CD16/32^{low}Lineage^{-}Sca\text{-}1^{-}c\text{-}kit^{+}$

Gra (granulocyte): $CD11b^{+}Gr\text{-}1^{+}B220^{-}CD3^{-}$

Mac (monocyte/macrophage): $CD11b^{+}Gr\text{-}1^{-}B220^{-}CD3^{-}$

Ery (erythroid progenitor): $CD71^{+}Ter119^{+}CD45^{+}$

B cell: $B220^{+}CD3^{-}CD11b^{-}Gr\text{-}1^{-}$

T cell: $CD3^{+}B220^{-}CD11b^{-}Gr\text{-}1^{-}$

Cell-cycle analysis

To analyze BrdU incorporation rates in vivo, mice were given an intraperitoneal injection of 100 mg/kg of BrdU (Sigma) in PBS and maintained on 1 mg/ml BrdU in the drinking water for three days. Bone marrow cells were incubated with biotinylated antibodies against lineage markers, followed by MojoSort Streptavidin Nanobeads (BioLegend), and $Lineage^{+}$ cells were depleted using a MojoSort Magnet (BioLegend). Frequencies of BrdU positive cells within the HSC compartment were measured by flow cytometry using the BrdU Flow Kit (BD Biosciences) with anti-BrdU antibody (3D4) conjugated with PE-Cy7 (BioLegend).

Human cord blood HSPC xenotransplantation

Anonymous human cord blood samples were obtained from the Department of Obstetrics and Gynecology at UConn Health. CD34-positive cells from each donor were enriched by Ficoll-Paque Premium density gradient media (1.078 g/mL density) followed by EasySep Human CD34 Positive Selection Kit II (STEMCELL Technologies) according to the manufacturer's instructions. Cell number and viability were assessed by AO/PI staining and counted by the LUNA-FL Dual Fluorescence Cell Counter. NBSGW recipient mice were transplanted with $5x10^4$ CD34-enriched cells via retro-orbital injection. Four months after transplantation, recipient mice were treated with mobilizing agents. Bone marrow cells were stained with anti-mouse CD45, anti-human CD45, anti-HLA-ABC, anti-human CD34, and anti-human CD38 antibodies. Peripheral blood cells are used for colony-forming assay. Derived colonies were stained with anti-mouse CD45, anti-human CD45, anti-human CD33, and anti-human CD235a antibodies.

Statistics

Data are shown as mean ± standard deviation (SD). The sample size used in each experiment was not formally justified for statistical power. No formal blinding was applied when performing the experiments or analyzing the data. Mice were allocated to experiments randomly and samples processed in an arbitrary order, but formal randomization techniques were not used. For analysis of the statistical significance of differences between two groups, we performed two-tailed unpaired Student's t-tests. For analysis of the differences among more than two groups, we performed one-way ANOVAs with Tukey's multiple comparisons test taking each cell population as one family. All statistical tests were performed using the GraphPad Prism software.

Supplementary Table 1. Antibodies used for flow-cytometry.

Antigen	Clone	Conjugation	Source	Catalog#	Identifier
BrdU	3D4	PE/Cyanine7	BioLegend	364118	RRID:AB_2814319
Human CD33	WM53	PE	BioLegend	303404	RRID:AB_314348
Human CD34	581	APC	BioLegend	343510	RRID:AB_1877153
Human CD38	HB-7	PE	BioLegend	356604	RRID:AB_2561900
Human CD45	HI30	FITC	BioLegend	304038	RRID:AB_2562050
Human CD235a	HIR2	PerCP/Cyanine5.5	BioLegend	306614	RRID:AB_10683170
Human HLA-A,B,C	W6/32	APC/Fire 750	BioLegend	311444	RRID:AB_2629629
Mouse CD2	RM2-5	Biotin	BioLegend	100104	RRID:AB_312651
Mouse CD3	17A2	Biotin	BioLegend	100244	RRID:AB_2563947
Mouse CD5	53-7.3	Biotin	BioLegend	100604	RRID:AB_312733
Mouse CD8α	53-6.7	Biotin	BioLegend	100704	RRID:AB_312743
Mouse/human CD11b	M1/70	APC	BioLegend	101212	RRID:AB_312795
Mouse CD16/32	93	Brilliant Violet 510	BioLegend	101333	RRID:AB_2563692
Mouse CD34	RAM34	FITC	BD Biosciences	553733	RRID:AB_1645242
Mouse CD45	30-F11	FITC	BioLegend	103108	RRID:AB_312973
Mouse CD45	30-F11	Alexa Fluor 700	BioLegend	103128	RRID:AB_493715
Mouse/human CD45R (B220)	RA3-6B2	Biotin	BioLegend	103204	RRID:AB_312989
Mouse/human CD45R (B220)	RA3-6B2	APC/Fire 750	BioLegend	103260	RRID:AB_2572109
Mouse CD48	HM48-1	PerCP/Cyanine5.5	BioLegend	103422	RRID:AB_2075051
Mouse CD48	HM48-1	Alexa Fluor 700	BioLegend	103426	RRID:AB_10612755
Mouse CD71	R17217	Brilliant Violet 421	BioLegend	113813	RRID:AB_10899739
Mouse CD117 (c-kit)	2B8	APC/Fire 750	BioLegend	105838	RRID:AB_2616739
Mouse CD127 (IL-7Rα)	A7R34	PE/Cyanine7	BioLegend	135014	RRID:AB_1937265
Mouse CD135 (Flt3)	A2F10	APC	BioLegend	135310	RRID:AB_2107050
Mouse CD150 (SLAM)	TC15-12F12.2	PE	BioLegend	115904	RRID:AB_313683
Mouse Ly-6A/E (Sca-1)	D7	Brilliant Violet 421	BioLegend	108128	RRID:AB_2563064
Mouse Ly-6A/E (Sca-1)	D7	PerCP/Cyanine5.5	BioLegend	108124	RRID:AB_893615
Mouse Ly-6G/Ly-6C (Gr-1)	RB6-8C5	Biotin	BioLegend	108404	RRID:AB_313369
Mouse Ly-6G/Ly-6C (Gr-1)	RB6-8C5	Brilliant Violet 510	BioLegend	108438	RRID:AB_2562215
Mouse Ter-119	TER-119	Biotin	BioLegend	116204	RRID:AB_313705
Mouse Ter-119	TER-119	PE/Cyanine7	BioLegend	116222	RRID:AB_2281408
Streptavidin		PE-CF594	BD Biosciences	562284	RRID:AB_11154598