ACS chemical biology

Letters

pubs.acs.org/acschemicalbiology

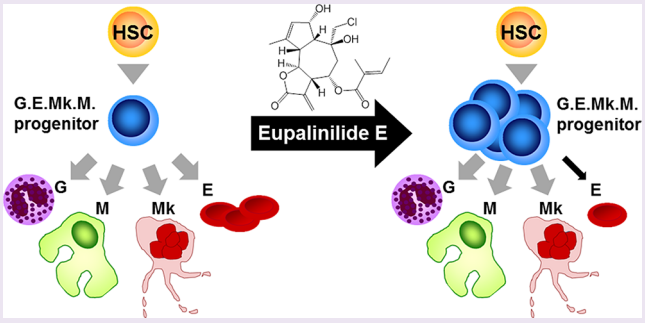
Eupalinilide E Inhibits Erythropoiesis and Promotes the Expansion of Hematopoietic Progenitor Cells

Lorenzo de Lichtervelde,[†] Anthony E. Boitano,[‡] Ying Wang,[§] Philipp Krastel,[§] Frank Petersen,[§] Michael P. Cooke,[‡] and Peter G. Schultz*[,†]

[†]Department of Chemistry, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, California 92037, United States
[‡]Genomics Institute of the Novartis Research Foundation, 10675 John Jay Hopkins Drive, San Diego, California 92121, United States
[§]Natural Products Unit, Novartis Institutes for Biomedical Research, Novartis Pharma AG, Switzerland

Supporting Information

ABSTRACT: Hematopoietic stem cells (HSCs) are the progenitor cells that give rise to all blood cells. The ability to control HSC differentiation has the potential to improve the success of bone marrow transplants and the production of functional blood cells *ex vivo*. Here we performed an unbiased screen using primary human CD34$^+$ hematopoietic stem and progenitor cells (HSPCs) to identify natural products that selectively control their differentiation. We identified a plant-derived natural product, eupalinilide E, that promotes the *ex vivo* expansion of HSPCs and hinders the *in vitro* development of erythrocytes. This activity was additive with aryl hydrocarbon receptor (AhR) antagonists, which are also known to expand HSCs and currently in clinical development. These findings reveal a new activity for eupalinilide E, and suggest that it may be a useful tool to probe the mechanisms of hematopoiesis and improve the *ex vivo* production of progenitors for therapeutic purposes.



Despite the enormous utility of natural products in research and medicine, their effects on stem cell fate have been relatively unexplored. The few examples that do exist, e.g., phorbol esters, which are widely used to induce macrophage differentiation,[1] suggest that natural products might be useful tools in stem cell biology. To this end, we have begun to explore the utility of natural products for the expansion of stem cells in their undifferentiated state. Many adult stem cells are difficult to isolate and not very proliferative, including muscle satellite cells,[2] pancreatic β cell precursors,[3] oligodendrocyte progenitors,[4] and hematopoietic stem cells (HSCs).[5] The latter cells are particularly relevant since HSC transplants are currently used to treat blood diseases, including leukemias and autoimmune diseases, and the success of a transplant directly correlates with the number of HSCs used. However, because HSCs differentiate spontaneously when placed in culture *ex vivo*, it is difficult to expand the number of cells prior to transplant. Overcoming this exhaustion phenomenon has long been a focus of regenerative medicine, but current protocols have had limited success and rely mostly on cytokines and other secreted factors.[6,7] We[8] and others[9] have focused on the identification of small drug-like molecules that control the self-renewal or selective differentiation of HSCs. Here we report the results of an imaged-based screen of a library of natural products using primary human CD34$^+$ cells to identify natural products that selectively differentiate or maintain HSCs.

Primary human CD34$^+$ cells isolated from mobilized peripheral blood (mPB) were seeded into 384-well plates (2500 cells per well) in medium optimized for their self-renewal, yet permissive to myelo-erythroid differentiation (serum free medium supplemented with thrombopoietin (TPO), stem cell factor (SCF), FLT3 ligand (Flt-3L), and interleukin-6 (IL-6)).[8,10] A Novartis library of 704 pure natural products from microbial and plant origin, selected to optimize chemotype diversity, was added at a final concentration of 1 μM. The cells were incubated for 7 days, at which point the cultures, consisting of a mixture of CD34$^+$ and differentiated cells, were analyzed by flow cytometry. The numbers and percentages of HSCs, hematopoietic stem and progenitor cells (HSPCs), and lineage-committed cells were determined based on their immunophenotype: THY1$^+$ (HSCs), THY1$^-$CD34$^+$ (HSCs and progenitors), and CD34$^-$ (lineage-committed cells). Using this assay, we identified the plant natural product eupalinilide E (Figure 1) as a promoter of HSPC self-renewal.

Treatment of CD34$^+$ cells with 600 nM eupalinilide E (EC_{50}, 210nM) did not change the total number of nucleated cells after 7 days of culture, but induced a 50% increase in the percentage of CD34$^+$ cells and a 2-fold increase in the number

Received: January 10, 2013
Accepted: February 26, 2013
Published: February 26, 2013

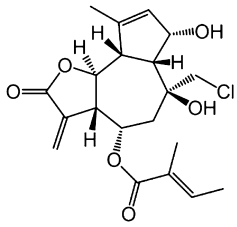


Figure 1. Structure of eupalinilide E.

of THY1$^+$ cells, two HSC cell surface markers (Figure 2A). To further evaluate the ability of eupalinilide to expand

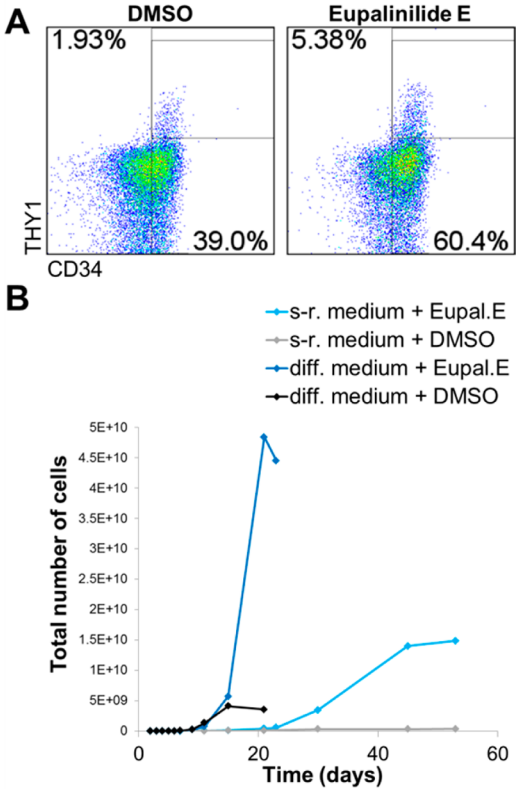


Figure 2. Eupalinilide E increases HSPC stemness markers and culture expansion. (A) MPB CD34$^+$ cells were cultivated for 7 days in self-renewal medium with vehicle (DMSO) or eupalinilide E (600nM). Total number of cells and numbers of CD34$^+$ and THY1$^+$ cells were measured by flow cytometry. (B) Cord blood CD34$^+$ cells were cultured in self-renewal medium or differentiation medium (self-renewal medium supplemented with 50 ng/mL EPO, GMCSF, GCSF, and IL3) and treated with DMSO or eupalinilide E (600nM). Cells were passaged regularly to maintain cell density below 1 million/mL, and total cell numbers were counted at regular intervals as indicated. Data shown are representative of at least 3 independent experiments.

hematopoietic progenitors *ex vivo*, we carried out long-term cultures of mPB and cord blood (CB) CD34$^+$ cells in self-renewal medium. As observed in the 7 day assay, eupalinilide E (600 nM) maintained a higher percentage of CD34$^+$ cells than vehicle-treated (0.1% DMSO) cultures, and although it did not completely prevent differentiation, it slowed the loss of this marker with time. In addition, although total cell numbers remained similar during the first 10 days of culture, eupalinilide-treated cultures significantly outgrew control cultures at longer times. Interestingly, the magnitude of this effect differed strongly between mPB (2-fold increase in total cell numbers after 18 days) and CB (4.5-fold increase after 18 days), with eupalinilide-treated CB cultures still growing after 40 days, whereas vehicle-treated cultures were exhausted and consisted of only differentiated cells. Consequently, after 45 days of CB culture, the total number of cells was 45-fold greater in eupalinilide E-treated cultures than vehicle-treated cultures (Figure 2B). In differentiation-inducing medium (self-renewal medium supplemented with four cytokines promoting differentiation: erythropoietin (EPO), granulocyte-macrophage colony stimulating factor (GMCSF), granulocyte colony stimulating factor (GCSF), and interleukin-3 (IL3)), CD34$^+$ cells proliferated slower in the presence of eupalinilide (600 nM), but whereas vehicle (0.1% DMSO) treated cultures exhausted before 15 days, eupalinilide-treated cultures grew past 20 days leading to a greater net expansion of cells (Figure 2B). These results support our hypothesis that the compound promotes the expansion of an early hematopoietic progenitor.

We next assessed the differentiation potential of eupalinilide E treated cells in colony-forming assays. MPB CD34+ cells were cultured in self-renewal medium with eupalinilide E (600 nM) or DMSO (0.1%). After 7 and 14 days of bulk culture, the resulting populations were transferred to semisolid differentiation medium (which promotes full myeloerythroid differentiation) for an additional 14 days, to allow for colony formation. Eupalinilide E-treated day 7 cultures generated 2.3-fold more total colonies compared to vehicle-treated cultures (Figure 3A). A comparable increase was observed in erythroid (E) and granulocyte-macrophage (GM) colonies and was prominent with GEMM colonies, which increased 19-fold (a difference visible macroscopically, see Supplementary Figure 2A). Day 14 cultures also showed a greater colony-forming potential when treated with eupalinilide E instead of vehicle, including a 3.6-fold increase in CFU-GM and 4.8-fold increase in CFU-E (Supplementary Figure 1A). Together, these results indicate that eupalinilide E promotes the maintenance of early, mixed-lineage progenitors in cell cultures. Importantly, this effect was also clear in cultures of cord blood (CB) CD34+ cells, where eupalinilide treatment (600 nM) led to a 13-fold increase in CFU-GEMM and 15-fold increase in CFU-GM after 14 days, relative to control (0.1% DMSO) cultures (Supplementary Figure 1B).

The results of the colony-forming assays further suggest that eupalinilide E does not alter the fate of hematopoietic progenitors irreversibly since CFUs in the eupalinilide-treated samples retained the capacity to proliferate and differentiate once inoculated in compound-free medium. However, in long-term cultures under differentiation conditions, the presence of eupalinilide E inhibits culture exhaustion and differentiation (Figure 2B). This latter result suggests that the outcome of the colony-forming assays might be different if eupalinilide E is present during the semisolid culture. To test this notion, mPB CD34+ cells were first cultured in self-renewal medium with 600 nM eupalinilide E. After 7 days, the compound was washed out, and cells from this single pool were then inoculated into either DMSO-treated (0.01%) or eupalinilide E-treated (600 nM) semisolid differentiation medium. Colonies were scored after 14 days of culture (Figure 3B). Importantly, the total number of colonies formed in the presence of eupalinilide and in the presence of vehicle was similar, further confirming that the compound does not inhibit the proliferation of CFUs. However, while numerous E and GEMM colonies formed in the presence of vehicle, these colonies formed in much lower numbers in the presence of eupalinilide E, suggesting that the

dx.doi.org/10.1021/cb4000234 | *ACS Chem. Biol.* 2013, 8, 866–870

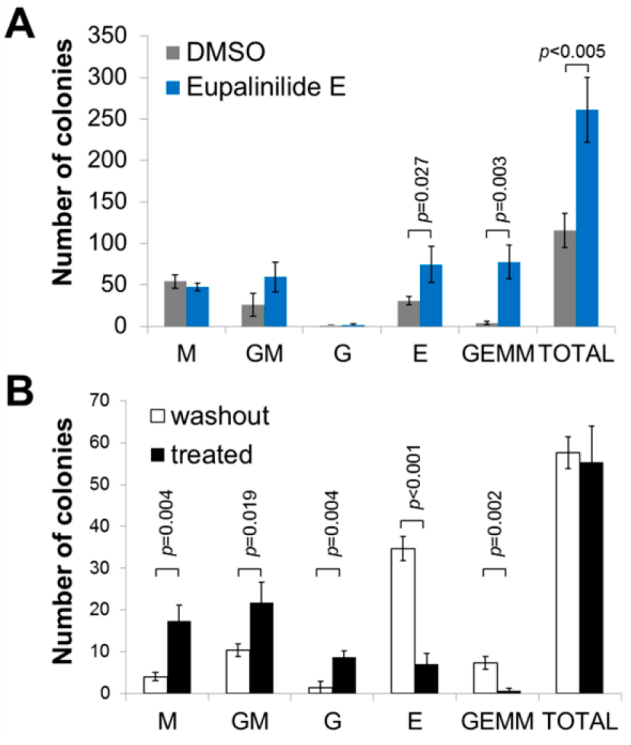


Figure 3. Eupalinilide E increases CFU-GEMM and CFU-E but inhibits the development of erythrocytes in HSPC cultures. (A) MPB $CD34^+$ cells were cultured for 7 days in self-renewal medium in the presence of 600 nM eupalinilide E or DMSO, then transferred into methylcellulose semisolid differentiation medium for colony formation. Colonies were scored after 14 days. (B) MPB $CD34^+$ cells were cultivated in self-renewal medium for 7 days with 600 nM eupalinilide E. Cells were then washed and inoculated into serum-free methylcellulose with or without eupalinilide E (600nM). Colonies were scored after 14 days. Data are represented as mean ± standard deviation. *p*-values were obtained with Student's *t*-test.

compound has an inhibitory effect on erythropoiesis. Instead, cells inoculated in the eupalinilide treated medium formed higher numbers of myeloid (M, GM, and G) colonies. Interestingly, M and GM colonies in eupalinilide-treated samples were also significantly larger and, unlike their counterparts from the untreated cultures, were easily visible with the naked eye (Supplementary Figure 2B).One explanation for this observation is that the compound increases the number of GEMM-colony forming progenitors in the culture but prevents these progenitors from differentiating down the erythrocyte lineage. As a result, large GEMM colonies form but do not acquire pigmentation and are therefore scored as GM. Alternatively, the block on erythroid differentiation may divert a larger number of these mixed-progenitors toward myeloid lineages. Together these results are consistent with the phenotype observed after 7 day cultures and support our hypothesis that eupalinilide E promotes the expansion of erythro-myeloid progenitors by generally slowing their differentiation and, at least in part, by blocking erythroid differentiation.

To further investigate the effects of eupalinilide E on the hematopoietic hierarchy, we tested whether the compound influences the emergence of different hematopoietic progenitors, including megakaryocyte-erythrocyte progenitors (MEPs, $CD34^+CD110^+$) and granulocyte-monocyte progenitors (GMPs, $CD34^+CD45ra^+$). Human mPB $CD34^+$ cells were cultured with 600 nM eupalinilide E or 0.1% DMSO, in self-renewal medium lacking TPO to prevent the endocytosis of CD110 (the TPO receptor). After 7 days in culture, the number of MEPs was 25% lower with eupalinilide E than in control cultures (Figure 4A). Simultaneously, cells expressing CD45RA decreased 20% in the presence of eupalinilide E (data not shown). The observation that the development of these two committed progenitors is inhibited by eupalinilide E is consistent with the colony forming assay results indicating that the progenitors expanded by eupalinilide possess dual erythroid-myeloid potential mand may bear more resemblance to common myeloid progenitors (CMP, $CD34^+CD45ra^-CD110^-$).

To determine the effects of eupalinilide on erythrocyte differentiation, we cultivated HSPCs in self-renewal medium supplemented with EPO, a cytokine critical for the survival and proliferation of erythroblasts. Analysis of markers specific for the erythrocyte lineage (CD71 and glycophorin A) revealed that although eupalinilide E did not prevent the development of erythroblasts ($CD71^+GlyA^{low}$ cells), it significantly decreased the percentage of terminally differentiated erythrocytes ($CD71^+GlyA^{hi}$ cells) after 7 days of culture (Figure 4B). Intriguingly, $CD71^+GlyA^-$ cells doubled in the presence of eupalinilide E. Moreover, under self-renewal conditions, eupalinilide E induced a 2.6-fold increase in $CD71^+$ cells after 7 days (Figure 4C,D). Although CD71, the transferrin receptor, is widely used as a marker for early erythrocytes, it is specifically expressed by dividing blood cells.[11,12] We therefore cannot exclude that the CD71 increase observed with eupalinilide E may not reflect an increase in early erythrocytes, but instead stem from an induction of proliferation by the compound, as we observed in long-term cultures in self-renewal medium. Together these results are consistent with the results of the colony forming assays and suggest that eupalinilide E inhibits erythropoiesis at two levels, MEP emergence and terminal erythrocyte maturation.

Finally, although the lack of synthetic routes to eupalinilide E hinders the generation of affinity probes for target identification, we investigated a number of potential cellular pathways that may be modulated by the compound. First, we determined whether the activity of eupalinilide E was dependent on the cytokines present in the self-renewal medium, which are known to play important roles in *ex vivo* HSC cultures. Surprisingly, the stem cell phenotype induced by eupalinilide E was independent of the 4 cytokines included in the self-renewing medium. The compound maintained higher percentages of CD34+ and THY1+ cells, regardless of the cytokines present in the medium, as long as one cytokine was present to allow cell survival and proliferation (Supplementary Figure 3).

Eupalinilide E is a sesquiterpene lactone natural product whose isolation was first reported in 2004 from the plant Eupatorium lindleyanum.[13] The only activity reported so far for this compound is cytotoxicity against P-388 and A-549 tumor cell lines. However, several sesquiterpene lactones have been shown to inhibit NF-κB signaling by alkylating cysteine residues in the NF-κB DNA-binding domain, leading to its inactivation.[14,15] We tested 4 sesquiterpene lactone analogues of eupalinilide E, some described as NF-κB inhibitors in the literature (Supplementary Figure 4) in the standard 7-day HSC differentiation assay. While the EC_{50} we observed for eupalinilide E is 211 nM (EC100 < 1 μM), none of the other compounds recapitulated eupalinilide's phenotype. Importantly, except for an analogue of cumambrin B, all

dx.doi.org/10.1021/cb4000234 | *ACS Chem. Biol.* 2013, 8, 866–870

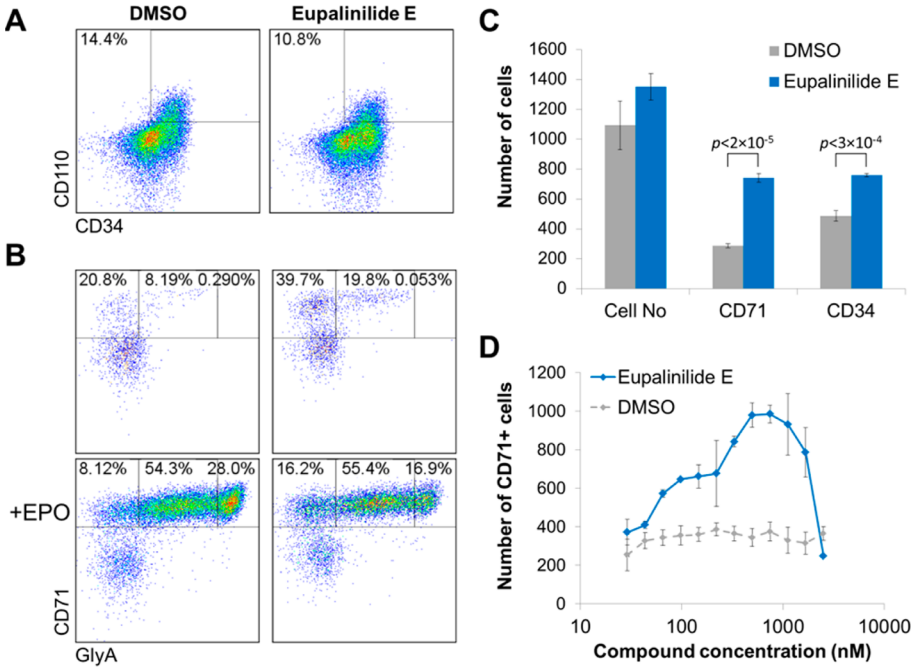


Figure 4. Eupalinilide E inhibits MEPs and terminal erythroid differentiation but induces an increase in CD71$^+$ cells. (A) MPB CD34$^+$ cells were plated in self-renewal medium lacking TPO and cultivated for 7 days with vehicle (DMSO) or eupalinilide E (600nM) to measure the production of MEPs (CD34$^+$CD110$^+$). (B) MPB CD34$^+$ cells were plated in self-renewal medium with or without EPO to stimulate erythropoiesis and cultivated for 7 days with vehicle (DMSO) or eupalinilide E (600nM). Expression of CD110, CD71, and glycophorin A was measured by flow cytometry. (C, D) Human mPB CD34$^+$ cells were cultivated for 7 days in self-renewal medium with vehicle (DMSO) or eupalinilide E (C, 600nM; D, indicated concentrations). Total number of cells and numbers of CD34$^+$, THY1$^+$, and CD71$^+$ cells were measured by flow cytometry. Data shown in panels A and B are representative of at least 3 independent experiments. Data in panels C and D are represented as mean ± standard deviation. *p*-values were obtained with Student's *t*-test.

sesquiterpene lactones tested showed cytotoxicity at the same concentration as eupalinilide E (starting around 1 μM), thereby excluding the possibility that the lack of activity of the analogues is due to an insufficient dose. These results suggest that the activity of eupalinilide E on HSPC differentiation is mediated by pathways other than NF-κB.

Antagonism of the aryl hydrocarbon receptor (AhR) also promotes HSPC expansion.[8] We therefore tested eupalinilide E in an AhR antagonism assay that measures the ability of the compound to inhibit the activation of an AhR-driven reporter construct triggered by dioxin (a potent AhR agonist). This reporter contains several dioxin response elements (DRE), sequences that are bound by AhR in the presence of TCDD (tetrachlorodibenzodioxin). Eupalinilide E did not inhibit DRE activity (Supplementary Figure 5A) even at concentrations equal to twice its EC100 (750 nM, see Figure 4D). Furthermore, the combination of eupalinilide E and the AhR antagonist SR1 had an additive effect on CD34$^+$ cell expansion (Supplementary Figure 5B), indicating that eupalinilide may be acting through a different pathway. Interestingly, the increase in CD71$^+$ cells induced by eupalinilide E was not observed with SR1, suggesting that eupalinilide E affects HSPC differentiation by a distinct mechanism.

A number of small molecules have been reported that increase HSPC expansion *ex vivo*, including AHR antagonists[8,16] and p38 inhibitors.[9] The effects of natural products on somatic stem cells, however, remain largely unexplored. A recent report described the *ex vivo* expansion of HSCs with garcinol,[17] a plant-derived histone acetyltransferase inhibitor. Here we report the identification of a plant natural product, eupalinilide E, as a promoter of the expansion of myelo-erythroid hematopoietic progenitors. Our results suggest that the compound acts by inhibiting differentiation, particularly to the erythrocyte lineage. Although the biological target of eupalinilide E remains unknown, this work highlights the ability of natural products to modulate stem cell biology.

METHODS

Chemicals. Eupalinilide E and sesquiterpene lactone analogues were obtained from the Chinese Institutes for Materia Medica through their collaboration with the Novartis Natural Products Unit. The isolation and purification of these compounds has been described previously.[13] TCDD was purchased from Crescent Chemical Co., Inc.

CD34$^+$ Cell Culture. All experiments were performed in HSC expansion media (StemSpan SFEM, StemCell Technologies) supplemented with 1× antibiotics and the following recombinant human cytokines: thrombopoietin, IL6, Flt3 ligand, and stem cell factor [100 ng/mL, R&D Systems]) unless otherwise indicated. Human mPB CD34$^+$ cells were purified from fresh human leukophoresed mobilized peripheral blood (AllCells) using direct CD34 progenitor cell isolation kits (Miltenyi Biotec) following manufacturer's protocols. Human CB cells were purified from fresh cord blood (Bioreclamation), using the same protocol.

Small Molecule Screen. CD34$^+$ cells were resuspended in HSC expansion medium (5×10^4 cells/mL) before being aliquoted in 384-well plates (Greiner Bio-One). Compounds were added immediately after plating. Cells were cultured at 37 °C in 5% CO_2.

Flow Cytometry. Cells were stained in staining medium (HBSS supplemented with FBS [2%] and EDTA [2 mM]) at 4 °C for 1 h with conjugated antibodies, then washed with staining medium and analyzed. Multicolor analysis for cell phenotyping was performed on a LSR II flow cytometer (Becton Dickinson). Antibodies used to detect human epitopes were PerCP anti-CD34 (BD Bioscience), APC anti-CD90 (BD Biosciences), PECy7 anti-CD45ra (eBiosciences), FITC anti-CD71 (BD Bioscience), and PE anti-GlyA (BD Bioscience).

dx.doi.org/10.1021/cb4000234 | *ACS Chem. Biol.* 2013, 8, 866–870

Colony Forming Assays. Human mobilized peripheral blood $CD34^+$ cells were cultured for 14 days in the presence of eupalinilide E or vehicle (DMSO) and passaged to keep the cultures between 2×10^5–1×10^6 cells/mL. After 14 days, 1/20 of the cultures were inoculated into serum-free methylcellulose containing SCF, interleukin 6, erythropoietin, interleukin 3, granulocyte, and granulocyte-macrophage colony stimulating factor (MethoCult SFH4436, StemCell Technologies), supplemented with 1× antibiotics, thrombopoietin, and Flt3 ligand, and distributed into three 35 mm dished. Colonies were scored after 14 days with an inverted microscope at 40× magnification. Numbers reported represent the average of the number of colonies scored from three dishes.

Cell Culture. All cytokines were purchased from R&D Systems. HEK293T cells were maintained in Dulbecco's modified Eagle's medium (Cellgro) containing 10% fetal bovine serum (Gibco), at 37 °C, in 5% CO_2.

Plasmid Constructs. pGudLuc was kindly provided by Prof. Gary Perdew.

Luciferase Reporter Assay. HEK293 cells were plated 10^4 per well of a 96-well plate. Cells were transfected with pGudLuc using Fugene6 (Roche). Cells were treated with various concentrations of eupalinilide E or DMSO with or without 3 nM TCDD and incubated overnight. Luciferase production was then measured using Bright Glo (Promega).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Supplementary Figures 1–5. This material is available free of charge *via* the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: Schultz@scripps.edu.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. F.P., P.K., and Y.W. designed and assembled the natural products library; A.E.B., L.d.L., M.P.C., and P.G.S. designed the research; L.d.L. performed the research; A.E.B., L.d.L., M.P.C., and P.G.S. analyzed data; and A.E.B., L.d.L., and P.G.S. wrote the paper.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to G. Perdew for kindly providing the pGudluc plasmid. We would like to thank Prof. YUE Jian Min from the Shanghai Institute of Materia Medica for the supply of eupalinilide E and sesquiterpene lactone analogs and Prof. ZHAO Wei Min from the Shanghai Institute of Materia Medica for the supply of Hyrcanin. This work was supported by The Skaggs Institute for Chemical Biology (PGS). This is manuscript #22073 of The Scripps Research Institute.

■ ABBREVIATIONS

HSC, hematopoietic stem cell; HSPC, hematopoietic stem and progenitor cell; SFEM, serum free expansion medium; EPO, erythropoietin; IL, interleukin; SCF, stem cell factor; TPO, thrombopoietin

■ REFERENCES

(1) Blumberg, P. M. (1988) Protein kinase-C as the receptor for the phorbol ester tumor promoters: 6th Rhoads Memorial Award Lecture. *Cancer Res. 48*, 1–8.

(2) Relaix, F., and Marcelle, C. (2009) Muscle stem cells. *Curr. Opin. Cell. Biol. 21*, 748–753.

(3) Borowiak, M., and Melton, D. A. (2009) How to make beta cells? *Curr. Opin. Cell. Biol. 21*, 727–732.

(4) Franklin, R. J., and Ffrench-Constant, C. (2008) Remyelination in the CNS: from biology to therapy. *Nat. Rev. Neurosci. 9*, 839–855.

(5) Chou, S., Chu, P., Hwang, W., and Lodish, H. (2010) Expansion of human cord blood hematopoietic stem cells for transplantation. *Cell Stem Cell 7*, 427–428.

(6) Zhang, C. C., Kaba, M., Ge, G., Xie, K., Tong, W., Hug, C., and Lodish, H. F. (2006) Angiopoietin-like proteins stimulate ex vivo expansion of hematopoietic stem cells. *Nat. Med. 12*, 240–245.

(7) Delaney, C., Heimfeld, S., Brashem-Stein, C., Voorhies, H., Manger, R. L., and Bernstein, I. D. (2010) Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. *Nat. Med. 16*, 232–236.

(8) Boitano, A. E., Wang, J., Romeo, R., Bouchez, L. C., Parker, A. E., Sutton, S. E., Walker, J. R., Flaveny, C. A., Perdew, G. H., Denison, M. S., Schultz, P. G., and Cooke, M. P. (2010) Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. *Science 329*, 1345–1348.

(9) Wang, Y., Kellner, J., Liu, L., and Zhou, D. (2011) Inhibition of p38 mitogen-activated protein kinase promotes ex vivo hematopoietic stem cell expansion. *Stem Cells Dev. 20*, 1143–1152.

(10) Murray, L. J., Young, J. C., Osborne, L. J., Luens, K. M., Scollay, R., and Hill, B. L. (1999) Thrombopoietin, flt3, and kit ligands together suppress apoptosis of human mobilized CD34(+) cells and recruit primitive CD34(+)Thy-1(+) cells into rapid division. *Exp. Hematol. 27*, 1019–1028.

(11) Judd, W., Poodry, C. A., and Strominger, J. L. (1980) Novel surface antigen expressed on dividing cells but absent from nondividing cells. *J. Exp. Med. 152*, 1430–1435.

(12) Trowbridge, I. S., and Omary, M. B. (1981) Human cell surface glycoprotein related to cell proliferation is the receptor for transferrin. *Proc. Natl. Acad. Sci. U.S.A. 78*, 3039–3043.

(13) Huo, J., Yang, S. P., Ding, J., and Yue, J. M. (2004) Cytotoxic sesquiterpene lactones from *Eupatorium lindleyanum*. *J. Nat. Prod. 67*, 1470–1475.

(14) Rungeler, P., Castro, V., Mora, G., Goren, N., Vichnewski, W., Pahl, H. L., Merfort, I., and Schmidt, T. J. (1999) Inhibition of transcription factor NF-κB by sesquiterpene lactones: a proposed molecular mechanism of action. *Bioorg. Med. Chem. 7*, 2343–2352.

(15) Siedle, B., Garcia-Pineres, A. J., Murillo, R., Schulte-Monting, J., Castro, V., Rungeler, P., Klaas, C. A., Da Costa, F. B., Kisiel, W., and Merfort, I. (2004) Quantitative structure–activity relationship of sesquiterpene lactones as inhibitors of the transcription factor NF-κB. *J. Med. Chem. 47*, 6042–6054.

(16) Bouchez, L. C., Boitano, A. E., de Lichtervelde, L., Romeo, R., Cooke, M. P., and Schultz, P. G. (2011) Small-molecule regulators of human stem cell self-renewal. *ChemBioChem 12*, 854–857.

(17) Nishino, T., Wang, C., Mochizuki-Kashio, M., Osawa, M., Nakauchi, H., and Iwama, A. (2011) Ex vivo expansion of human hematopoietic stem cells by garcinol, a potent inhibitor of histone acetyltransferase. *PLoS One 6*, e24298.