Histone deacetylase inhibition modulates cell fate decisions during myeloid differentiation

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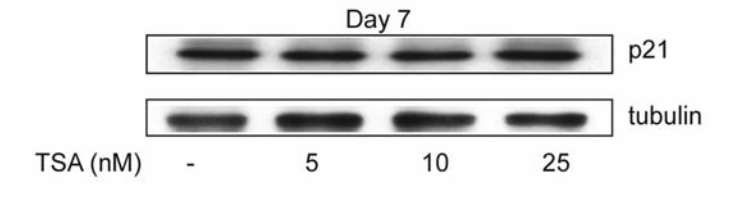
Online Supplementary Appendix

Isolation and culture of human CD34⁺ cells

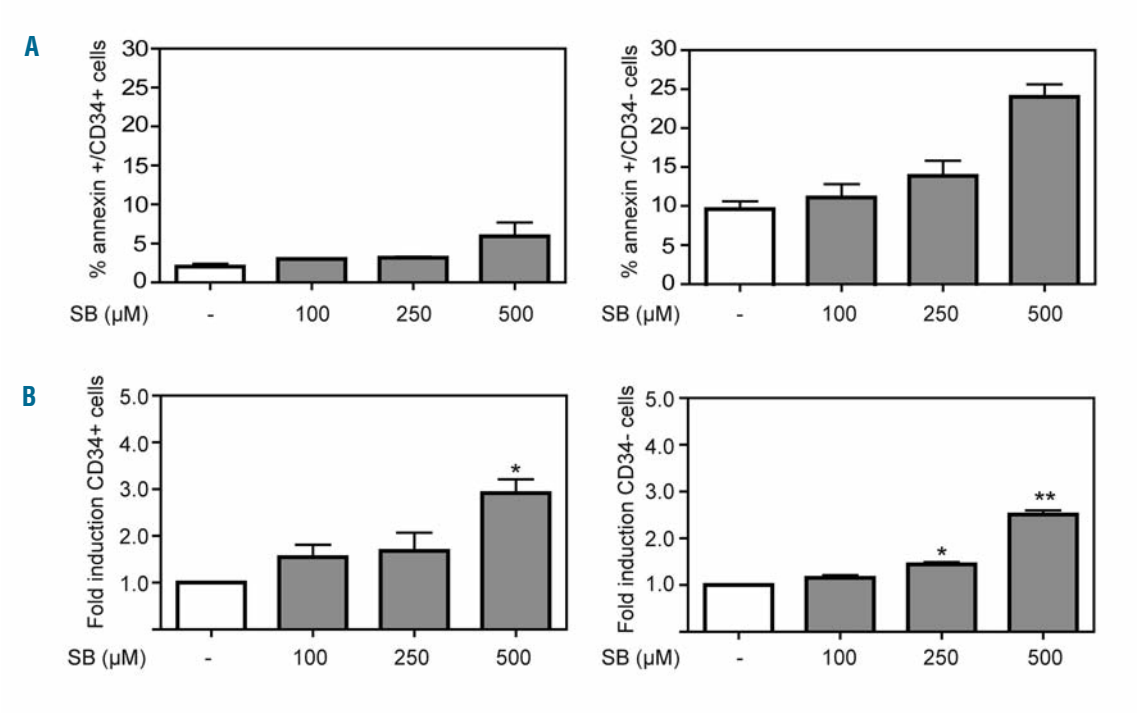
Mononuclear cells were isolated from human umbilical cord blood by density centrifugation over a Ficoll-Paque solution (density 1.077 g/mL). MACS immunomagnetic cell separation (Miltenyi Biotech, Auburn, CA, USA) using a hapten-conjugated antibody against CD34, which was coupled to beads, was used to isolate $CD34^+$ cells. $CD34^+$ cells ($5.0\text{-}6.0\times10^4$) were cultured in Iscove's modified Dulbecco's medium (Gibco, Paisley, UK) supplemented with 8% fetal calf serum (FCS) (Hyclone, South Logan, UT, USA), 50 μmol/L of ß-mercaptoethanol, 10 U/mL of penicillin, 10 μg/mL of streptomycin, and 2 mM glutamine at a density of 0.3×10^6 cells/mL. Cells were differentiated towards neutrophils in 17 days upon addition of stem cell factor (SCF) (50 ng/mL), FLT-3 ligand (50 ng/mL), granulocyte macrophage colony-stimulating factor (GM-CSF) (0.1 nmol/L), interleukin 3 (IL-3) (0.1 nmol/L), and granulocyte colony-stimulating factor (G-CSF) (30 ng/mL). Every 3 days, cells were counted with trypan blue, and fresh medium was added to a density of 5.0×10^5 cells/mL. After 3 days of differentiation only G-CSF was added to the cells. The HDAC inhibitors trichostatin A (TSA), sodium butyrate (SB) and valproic acid (VPA) (Alexis Chemicals, Lausen, Switzerland) were added to the fresh medium every 3 days. Umbilical cord blood was collected after informed consent was provided according to the Declaration of Helsinki. Protocols were approved by the ethics committee of the University Medical Center Utrecht.

Flowcytometric analysis of the myeloid progenitor compartment

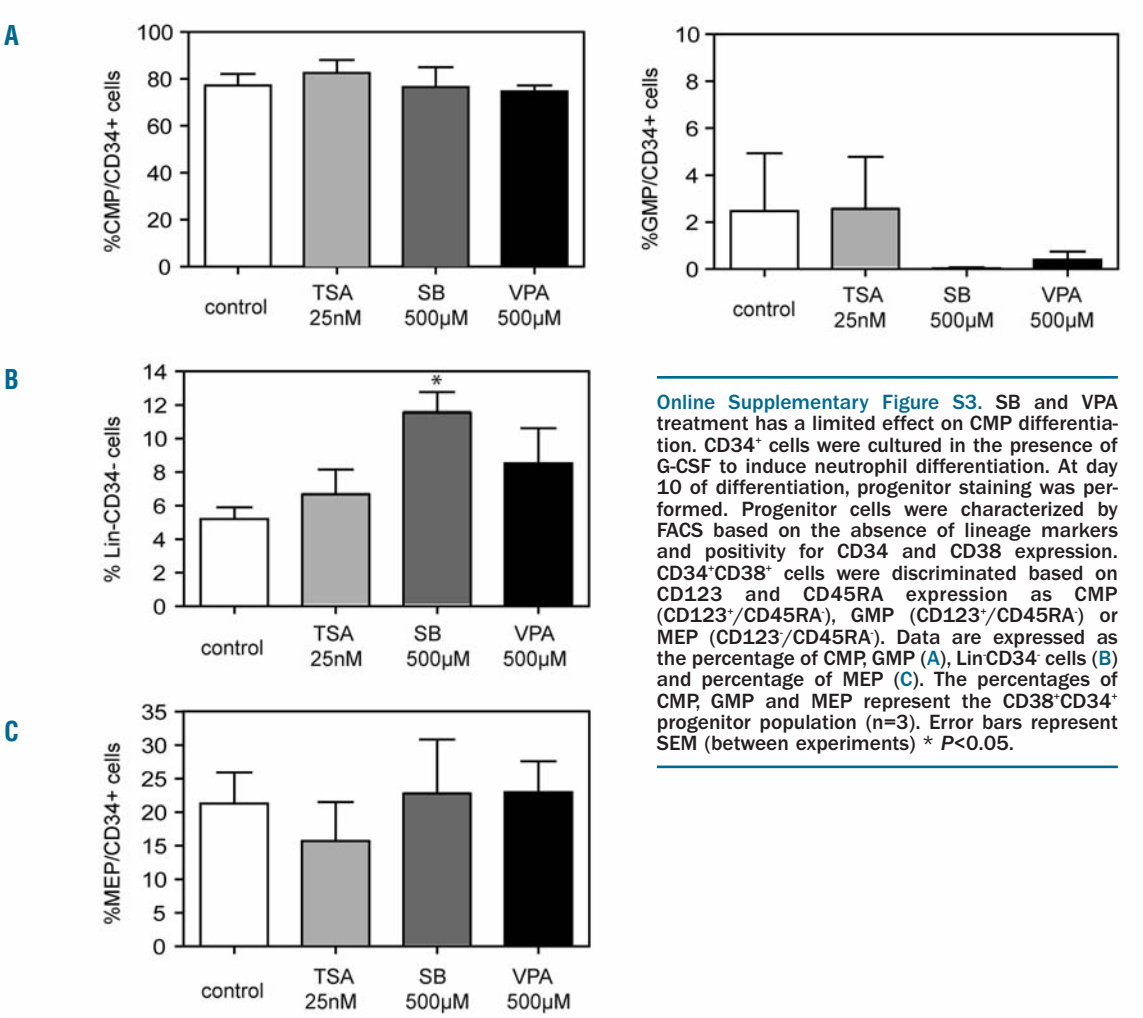
$CD34^+$ cells were isolated and cultured to induce neutrophil differentiation as described above. At days 3, 7 and 10 of differentiation, cells were washed and resuspended in PBS/5% FCS (Hyclone) and incubated for 30 min on ice with a mixture of antibodies (all from Becton Dickinson). Lineage markers included CD2, CD3, CD4, CD7, CD8, CD14, and CD235a and myeloid progenitors are negative for these markers. The lineage negative (Lin^-), $CD34^+CD38^-$ population consists of hematopoietic stem cells (HSC). $Lin^-CD34^+CD38^+CD123^+CD45RA^-$ cells are CMP, whereas $Lin^-CD34^+CD38^+CD123^+CD45RA^+$ cells are granulocyte-macrophage progenitors (GMP). The $Lin^-CD34^+CD38^+CD123^-CD45RA^-$ cell population contains the megakaryocyte-erythroid progenitors (MEP). Cell populations containing HSC, CMP, GMP and MEP were characterized by FACS analysis (FACS Canto, Becton Dickinson). Isotype antibody staining was used to ensure gating of the correct population.



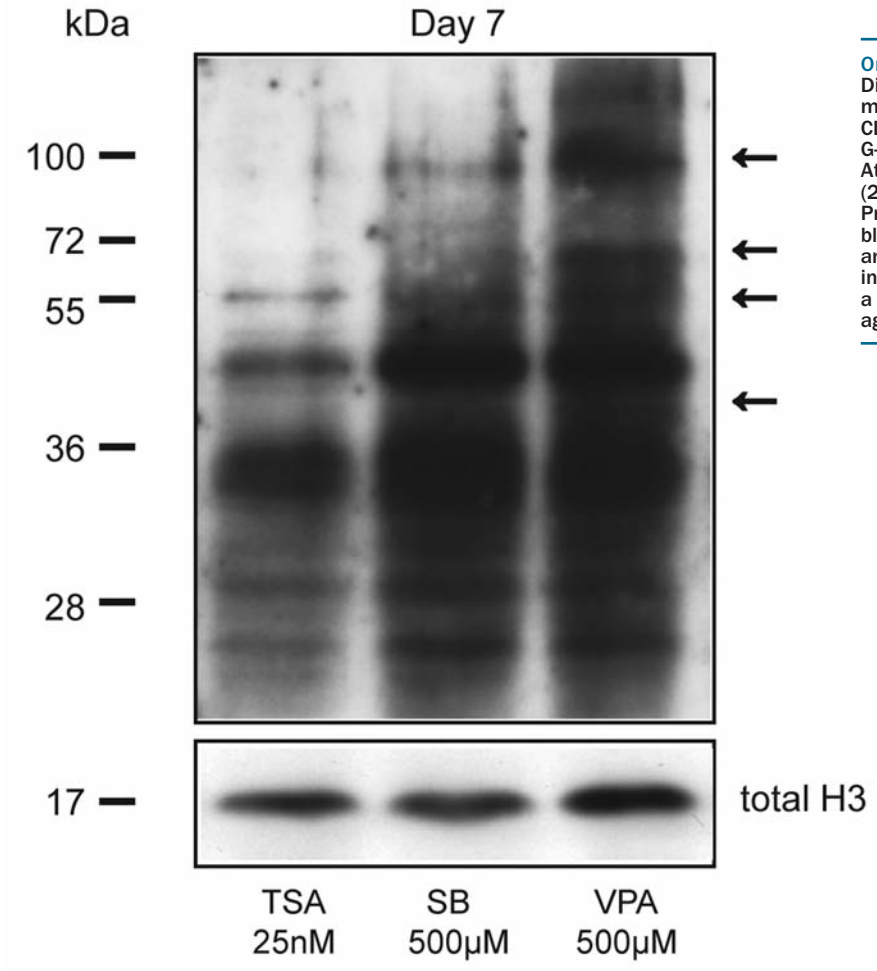
Online Supplementary Figure S1. TSA treatment of neutrophil progenitors is not associated with increased $p21^{cip1}$ expression. $CD34^+$ cells were cultured in the presence of G-CSF to induce neutrophil differentiation. At day 7 of differentiation, protein lysates were prepared and western blot analysis was performed with an antibody against p21. As a control for equal loading, an antibody against tubulin was used (n=2).



Online Supplementary Figure S2. CD34-negative cells and CD34-positive cells are both susceptible to SB-induced apoptosis. $CD34^+$ cells were cultured in the presence of G-CSF to induce neutrophil differentiation. At day 7 of differentiation, apoptosis was measured by annexin-V staining and $CD34^+$ expression was analyzed by FACS. Data are expressed as the percentage of annexin V-positive/CD34-positive and annexin V-positive/CD34-negative cells (A) and as the fold induction in apoptosis in CD34-positive and CD34-negative cells (B) (n=2). Error bars represent SEM (between experiments) * $P<0.05$, ** $P<0.01$.



Online Supplementary Figure S3. SB and VPA treatment has a limited effect on CMP differentiation. $CD34^+$ cells were cultured in the presence of G-CSF to induce neutrophil differentiation. At day 10 of differentiation, progenitor staining was performed. Progenitor cells were characterized by FACS based on the absence of lineage markers and positivity for CD34 and CD38 expression. $CD34^+CD38^+$ cells were discriminated based on CD123 and CD45RA expression as CMP ($CD123^+/CD45RA^-$), GMP ($CD123^+/CD45RA^-$) or MEP ($CD123^-/CD45RA^-$). Data are expressed as the percentage of CMP, GMP (A), Lin^-CD34^- cells (B) and percentage of MEP (C). The percentages of CMP, GMP and MEP represent the $CD38^+CD34^+$ progenitor population (n=3). Error bars represent SEM (between experiments) * $P<0.05$.



Online Supplementary Figure S4. Differential effects of HDAC inhibitor treatment on non-histone protein acetylation. $CD34^+$ cells were cultured in the presence of G-CSF to induce neutrophil differentiation. At day 7, cells were treated for 8 h with TSA (25 nM), SB (500 μM) or VPA (500 μM) Protein lysates were prepared and western blot analysis was performed utilizing an antibody against acetylated lysines. Arrows indicate HDAC inhibitor-specific effects. As a control for equal loading an antibody against total H3 was used (n=2).