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A Single Metabolite which Modulates Lipid Metabolism Alters Hematopoietic Stem/Progenitor Cell Behavior and Promotes Lymphoid Reconstitution

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SUMMARY

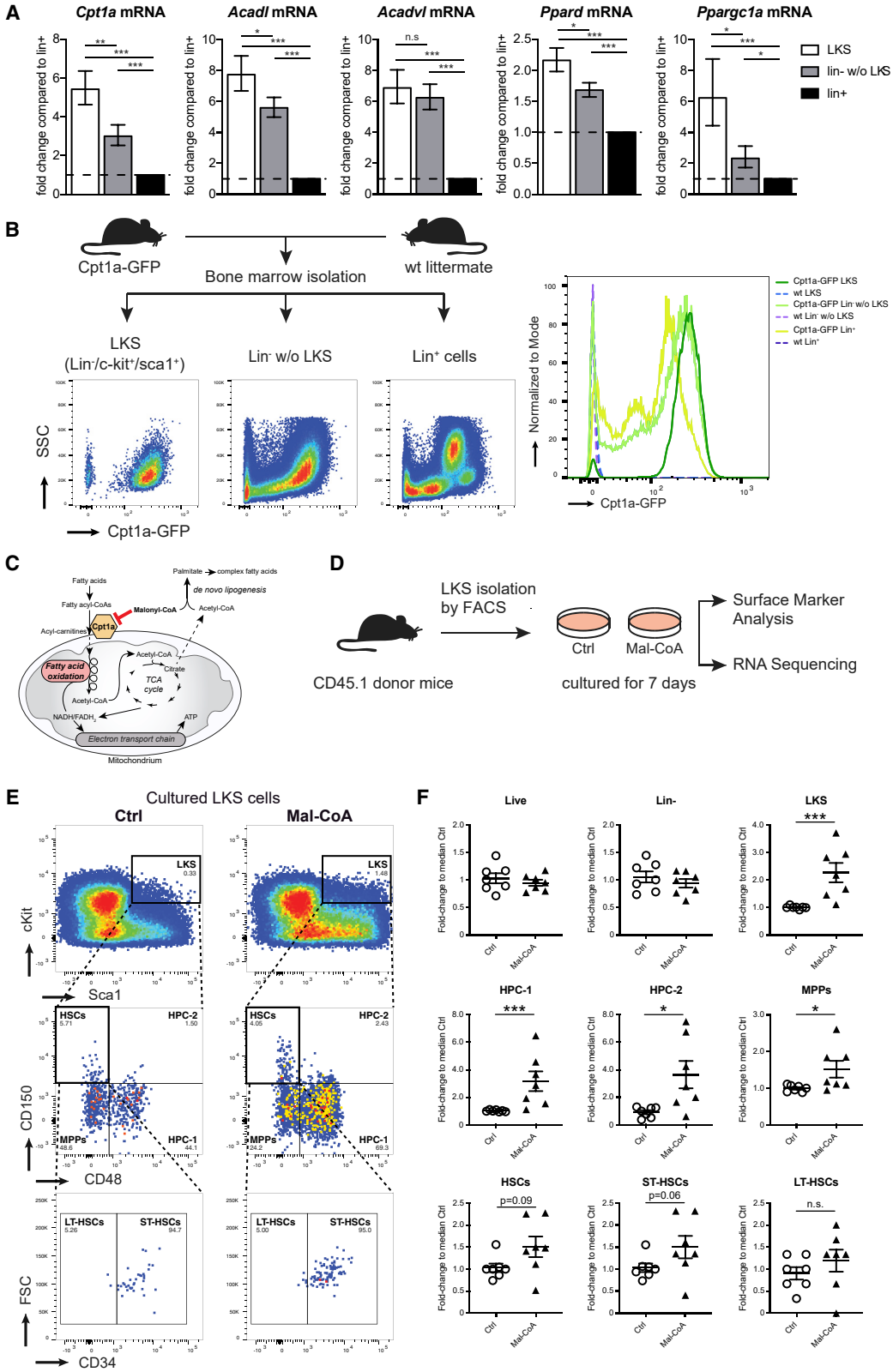
Fatty acid β-oxidation (FAO), the breakdown of lipids, is a metabolic pathway used by various stem cells. FAO levels are generally high during quiescence and downregulated with proliferation. The endogenous metabolite malonyl-CoA modulates lipid metabolism as a reversible FAO inhibitor and as a substrate for *de novo* lipogenesis. Here we assessed whether malonyl-CoA can be exploited to steer the behavior of hematopoietic stem/progenitor cells (HSPCs), quiescent stem cells of clinical relevance. Treatment of mouse HSPCs *in vitro* with malonyl-CoA increases HSPC numbers compared with nontreated controls and ameliorates blood reconstitution capacity when transplanted *in vivo*, mainly through enhanced lymphoid reconstitution. Similarly, human HSPC numbers also increase upon malonyl-CoA treatment *in vitro*. These data corroborate that lipid metabolism can be targeted to direct cell fate and stem cell proliferation. Physiological modulation of metabolic pathways, rather than genetic or pharmacological inhibition, provides unique perspectives for stem cell manipulations in health and disease.

INTRODUCTION

Cellular metabolism has emerged as a key mechanism regulating stem cell quiescence and proliferation (Chandel et al., 2016; Ito and Suda, 2014) and lipid metabolism plays an important role in this regulation. Whereas the build-up of lipids via *de novo* lipogenesis promotes proliferation of stem cells (Knobloch et al., 2013; Wang et al., 2017), the breakdown of lipids via fatty acid β-oxidation (FAO) is crucial for the maintenance of adult stem cells, such as neural stem cells (Knobloch et al., 2017; Stoll et al., 2015), muscle satellite cells (Ryall et al., 2015), intestinal stem cells (Mihaylova et al., 2018), and hematopoietic stem cells (Ito et al., 2012), indicating conserved regulatory mechanisms of stem cell activity through metabolism.

The role of FAO in stem cell maintenance has been established by genetic manipulation of FAO enzymes, such as the peroxisome-proliferator activated receptors (PPARs) or carnitine palmitoyl transferase 1 (Cpt1), or via pharmacological inhibitors and activators of FAO. Although these approaches are well-suited to test the importance of a metabolic pathway, they may not reflect the changes that occur under physiological conditions. For instance, cells are more likely shifting between different metabolic pathways rather than switching a pathway on or off completely. A shift rather than switch concept is in line with recent single-cell RNA sequencing, data suggesting transitions from one state to the other rather than clear stepwise progressions (Macaulay et al., 2016; Shin et al., 2015).

We have shown that shifting lipid metabolism using malonyl-CoA, the endogenous metabolite that reversibly inhibits Cpt1a/FAO (Foster, 2012) and serves as a building block for *de novo* lipogenesis, alters the behavior of quiescent neural stem/progenitor cells (NSPCs). The addition of malonyl-CoA to cultured quiescent NSPCs is sufficient to induce proliferation (Knobloch et al., 2017). Here we addressed whether malonyl-CoA has similar effects on HSPCs. Increasing the numbers of HSPCs *in vitro,* without losing stem cell capacity, is of clinical interest, specifically for umbilical cord blood (Kiernan et al., 2017) and for expansion of HSPCs after genetic modifications, as required for gene therapy approaches (Morgan et al., 2017). In addition, as HSPC function is compromised with aging (Kovtonyuk et al., 2016), increasing HSPC function in the aging context is of great interest. Previously, Ito and colleagues had shown the importance of FAO for HSPC maintenance, such that a complete block of FAO led to HSPC exhaustion (Ito et al., 2012). Interestingly, they noted that this block of FAO led initially to a short-term expansion of HSPCs. Given previous data on quiescence exit upon FAO modulation in NSPCs (Knobloch et al., 2017), we thus rationalized that modulating lipid metabolism through malonyl-CoA could expand



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HSPCs without leading to exhaustion. We therefore cultured HSPCs from young and old mice and human cord blood with or without malonyl-CoA *in vitro* and analyzed their behavior.

RESULTS

HSPCs Show High Expression of Genes Involved in FAO

We first measured the expression of key FAO genes by qRT-PCR in three different cell populations derived from the bone marrow (BM): an HSPCs enriched population (Lin$^-$cKit$^+$Sca1$^+$ cells, LKS cells), an intermediate population (lineage negative cells without LKS, Lin$^-$ w/o LKS) and a population consisting of differentiated progeny (lineage positive cell population, Lin$^+$ cells) separated by flow cytometry (Figure S1A). Carnitine palmitoyltransferase 1a (*Cpt1a*), a key player in FAO as well as other FAO enzymes and regulators were highly upregulated in LKS cells compared with Lin$^+$ cells (Figure 1A), suggesting that FAO is a pathway specifically important in HSPCs. These findings are in line with previous findings showing an important role for FAO and Ppar-δ in HSPC maintenance (Ito et al., 2012).

Given the high expression of Cpt1a in the LKS population, we asked whether Cpt1a could be used as an HSPC specific marker. We used a reporter mouse expressing GFP under the Cpt1a promoter (Cpt1a-GFP, Gong et al., 2003), isolating the same three cell populations and comparing them with wild-type cells. Indeed, a clear GFP signal was detected in cells from Cpt1a-GFP mice, highest in the LKS population followed by Lin$^-$ w/o LKS and Lin$^+$ (Figure 1B), confirming the gene expression data. We next subdivided the LKS population into five subgroups based on the expression of the surface markers CD150, CD48, and CD34 (Kiel et al., 2005; Oguro et al., 2013): Long-term (LT)-/short-term (ST)-HSCs are defined as LKS CD150$^+$CD48$^-$CD34$^{-\text{ or }+}$, two closely related progenitors termed HPC-1/HPC-2 as LKS CD150$^{-\text{ or }+}$ CD48$^+$ and multipotent progenitors (MPPs) as LKS CD150$^-$CD48$^-$ (Figure S1B). However, all these subgroups of the LKS population showed equally high levels of GFP expression (Figure S1C). Thus, using Cpt1a-GFP mice, a further distinction between these purified populations within the LKS population was not possible.

Malonyl-CoA Addition *In Vitro* Alters HSPC Behavior

We next asked whether modulation of FAO may alter HSPC behavior. The metabolite malonyl-CoA acts at the same time as the endogenous inhibitor of Cpt1a/FAO and as a rate-limiting substrate for *de novo* lipogenesis (Figure 1C), providing an endogenous mechanism to balance the build-up and breakdown of lipids within a cell (Foster, 2012). Thus, increasing the levels of malonyl-CoA provides an experimental opportunity to manipulate lipid metabolism. In line with previous results (Knobloch et al., 2017), we further validated malonyl-CoA uptake in NSPCs (Figures S1D–S1G). Taken together, these data, established in NSPCs, suggest that extracellular malonyl-CoA can be taken up and that it modulates FAO, although in a much milder way than pharmacological FAO inhibitors such as Etomoxir.

Therefore, we tested the effect of malonyl-CoA on purified ST- and LT-HSCs, using two different doses (5 and 50 μM) over 7 days in culture. Proliferation kinetics were assessed through analysis of brightfield images. Both ST-HSCs and LT-HSCs reacted to malonyl-CoA with increased numbers (Figures S2A–S2J). Due to observed changes in both HSC populations, we next tested the effect of malonyl-CoA on a population comprising LT-, ST-HSCs and progenitor cells, namely LKS cells. We cultured freshly isolated LKS cells *in vitro* with or without 5, 50, or 100 μM malonyl-CoA (Figure S2K); 100 μM resulted in the overall highest cell number and was used for all following experiments. After 7 days in

Figure 1. FAO Is High in HSPCs and Exposure to Malonyl-CoA Alters HSPC Behavior *In Vitro*

(A) mRNA expression (fold change ±SEM) of key FAO genes is upregulated in freshly isolated LKS cells compared with an intermediate cell population (Lin$^-$ w/o LKS) and differentiated progeny (Lin$^+$). Shown are carnitine palmitoyl transferase 1a (*Cpt1a*), long-chain acyl-CoA dehydrogenase (*Acadl*), very long-chain acyl-CoA dehydrogenase (*Acadvl*), peroxisome proliferator-activated receptor beta/delta (*Ppard*) and the peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*Ppargc1a*). (n = 3, individual wells per condition) *p < 0.05; **p < 0.01; ***p < 0.001.

(B) Representative flow cytometry plots and GFP histograms show Cpt1a-GFP levels highest in the LKS population followed by Lin$^-$ w/o LKS and Lin$^+$ populations.

(C) Scheme depicting the dual function of malonyl-CoA as an endogenous Cpt1a inhibitor and a substrate of *de novo* lipogenesis.

(D) Schematic outline of the isolation, cultivation, and analysis of LKS cells with or without 100 μM malonyl-CoA.

(E) Surface marker expression of culture LKS cells was analyzed by flow cytometry.

(F) Quantification of the flow cytometry analysis. Total number of cells were normalized to the median of the corresponding control condition. Malonyl-CoA expanded the number of LKS cells with an increase in all the stem cell sub-populations. Statistical analysis was done including technical replicates by using nonparametric Mann-Whitney test (n = 7, individual wells per condition, two independent experiments, cells isolated from 2 × 6 pooled mice). *p < 0.05; **p < 0.01; ***p < 0.001. The increase in LKS cells upon malonyl-CoA exposure was also observed in several independent experiments with a different experimental setup, see Figures 3B and S2K.

culture we harvested the entire progeny of cultured LKS and performed surface marker analysis and RNA sequencing (Figures 1D and S2L). Malonyl-CoA addition significantly increased the total number of cells with LKS surface marker characteristics, without a change in the total number of live cells and Lin^- cells, nor a change in the percentage of Lin^- cells (Figures 1E and 1F). This increase was due to an increased total number of cells in all stem/progenitor subpopulations (HPC-1, HPC-2, MPPs, ST-HSCs, LT-HSCs), suggesting that the manipulation of lipid metabolism with the endogenous metabolite malonyl-CoA increases the total number of HSPCs *in vitro* (Figure 1F).

RNA sequencing after 7 days in culture (Figure 1D) revealed only small changes in gene expression with malonyl-CoA (123 genes); however, hierarchical clustering and heatmap display of these 123 genes (Figures 2A and 2B) revealed a clear separation of the HSPCs according to the treatment. Surprisingly, gene ontology (GO) enrichment analysis showed a majority of GO terms involved in immune system processes (Figure 2C), suggesting that exposure to malonyl-CoA might also influence HSPC fate.

Malonyl-CoA Treatment Increases HSPC Functionality *In Vivo*, Mainly by Enhancing Lymphoid Differentiation Potential

Next, we evaluated whether malonyl-CoA leads to enhanced HSPC maintenance and function *in vivo*. We transplanted the progeny of LKS cells cultured in the presence or absence of 100 μM malonyl-CoA for 7 days into lethally irradiated mice, and assessed their contribution to the myeloid and lymphoid lineage. Cultured LKS cells (with or without malonyl-CoA) were co-transplanted with freshly isolated BM cells in lethally irradiated recipient animals and we used congenic markers to distinguish the different cell sources (donor CD45.1, competitor CD45.1/2, recipient CD45.2) (Figure S3A). Blood composition was assessed at the indicated time points by flow cytometry (Figures 2D, S3A, and S3B). LKS cells exposed *in vitro* to malonyl-CoA showed a higher total engraftment potential compared with control LKS grown without malonyl-CoA (Figure 2E). Interestingly, primarily the reconstitution of the lymphoid lineage was significantly increased, while the myeloid lineage showed only a slight, nonsignificant increase (Figure 2E). BM analysis of the primary recipients 27-weeks post transplantation confirmed the increased contribution of malonyl-CoA–treated LKS cells to the lymphoid lineage (Figure 2F). Secondary recipients, who received BM from primary recipients injected with malonyl-CoA–treated LKS (Figure S3C), showed a slight increase in the overall total long-term repopulation capacity of the initial donor cells, primarily due to better lymphoid reconstitution (Figure S3D); however, the differences were not statistically significant.

In summary, malonyl-CoA-treated LKS cells engrafted overall better than control LKS and showed the capability to sustain long-term blood production in primary recipient mice. The observed increase in the reconstituted lymphoid population suggests that malonyl-CoA might lead to a biased fate preference toward the lymphoid lineage. The result that malonyl-CoA–treated LKS cells were slightly better than nontreated LKS cells in the secondary transplantation indicates that a modulation of lipid metabolism with malonyl-CoA does not lead to HSPC exhaustion.

Several Cell Types of the Lymphoid Lineage Are Increased upon Malonyl-CoA Treatment

To determine whether there is a similar bias toward the lymphoid fate *in vitro*, we performed high-dimensional flow cytometry on LKS progeny after 7 days in culture with malonyl-CoA, using 21 different surface markers (Figure 3A, Figures S4A and S4B). In addition to the increased number of LKS cells upon malonyl-CoA treatment (Figures 3B and 1F), we found that also lymphoid progenitors such as multipotent progenitor population 4 (MPP4s, similar to the HPC-1 population, Pietras et al., 2015) and common lymphoid progenitors (CLPs) were upregulated. Conversely, numbers of common myeloid progenitors (CMPs) were not altered (Figure 3B). The shift toward enhanced lymphoid commitment was also evident in further differentiated populations such as pre B cells, natural killer T (NKT) cells, and T cells ($CD3^+$, but $CD4^-$ and $CD8^-$), which were all significantly upregulated with malonyl-CoA compared with control LKS cells (Figure 3C). In contrast, myeloid progenitors including megakaryocyte/erythrocyte progenitors (MEPs), granulocyte/monocyte progenitors (GMPs), granulocyte progenitors (GPs), common monocyte progenitors (cMOPs), common DC progenitors (CDPs), and monocyte-DC progenitors (MDPs) were not affected by malonyl-CoA (Figure S4C), suggesting a selective differentiation toward the lymphoid lineage through manipulation of lipid metabolism. However, neutrophils were also significantly increased with malonyl-CoA, suggesting an independent action on the survival/proliferation of more mature populations (Figure S4C).

Taken together, this analysis confirmed a lymphoid bias upon malonyl-CoA treatment, similar to the results seen with *in vivo* transplantation (Figure 2E).

HSPCs From Aged Mice Remain Responsive to Malonyl-CoA Treatment

Aged mice have a strong bias toward myeloid lineage output, with negative consequences for immune function (Kovtonyuk et al., 2016). By using high-dimensional flow cytometry, we asked whether HSPCs from old mice remain responsive to malonyl-CoA (Figure 3D) with a similar lymphoid fate shift as seen in HSPCs from young mice (Figures 3B and 3C). The number of LKS cells from old mice increased

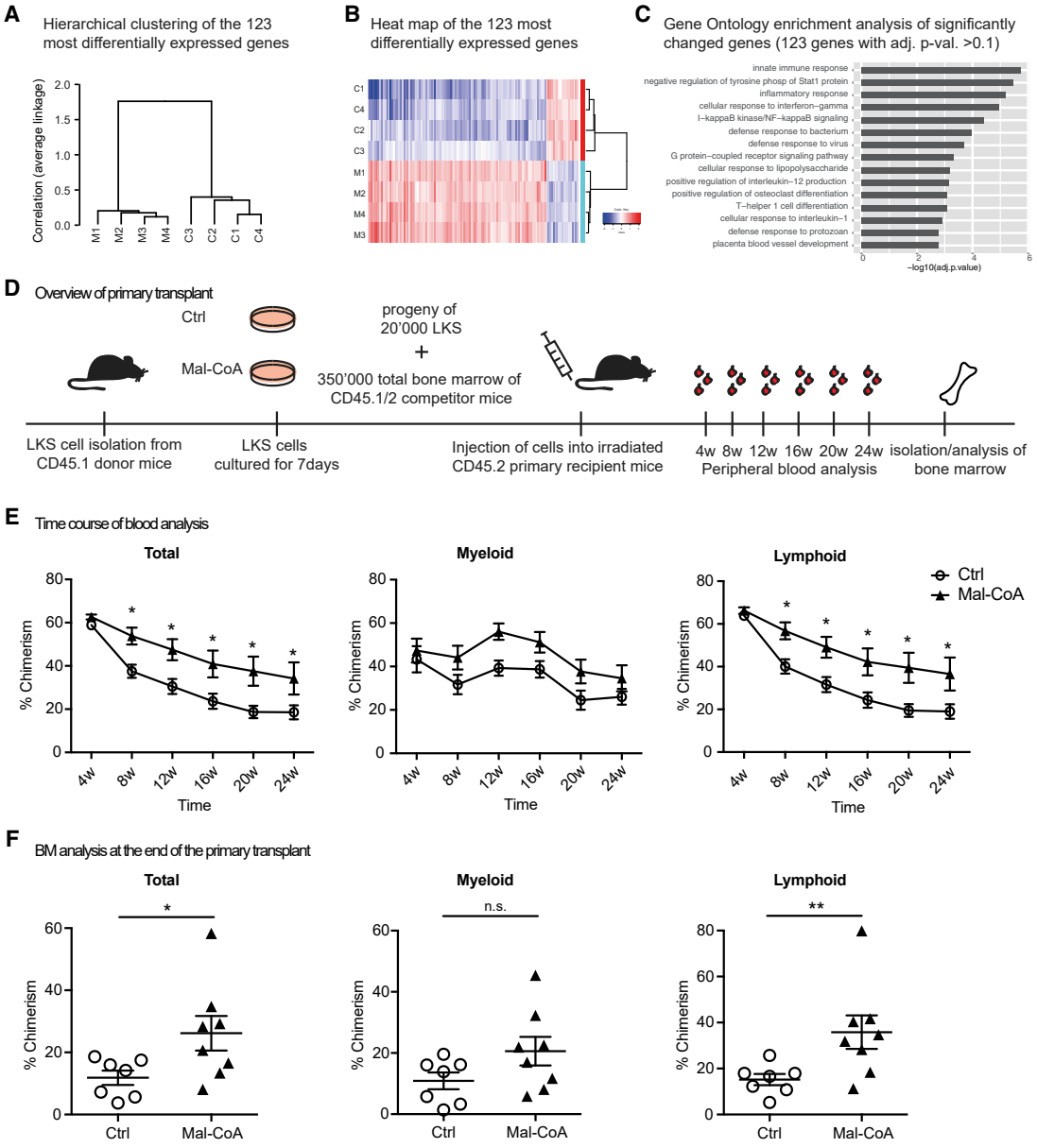

Figure 2. Malonyl-CoA Treatment Increases HSPC Functionality *In Vivo*, Primarily by Enhancing Lymphoid Differentiation Potential

(A) RNA sequencing revealed a hierarchical clustering into the two treatment groups, based on the 123 most differentially expressed genes.

(B) Heatmap representation.

(C) Gene ontology (GO) analysis of changed genes points toward increased immune system processes.

(D) Schematic outline of the primary transplantation set up.

(E) Blood analysis of transplanted mice by flow cytometry at different time points (4, 8, 12, 16, 20, 24 weeks posttransplant). Graphs display the percentage of CD45.1 cells among total cells. Statistical analysis is done with two-way ANOVA followed by Holm-Sidak's multiple comparison test (Ctrl n = 7, malonyl-CoA n = 8, number of mice per condition) *p < 0.05.

(F) Flow cytometry analysis of BM chimerism 27 weeks posttransplantation. Malonyl-CoA–treated LKS in the BM showed a significant increase in the engraftment in the total and lymphoid lineage, while the myeloid lineage was not significantly different. Statistical analysis was done using nonparametric Mann-Whitney test (Ctrl n = 7, malonyl-CoA n = 8, number of mice per condition). *p < 0.05.

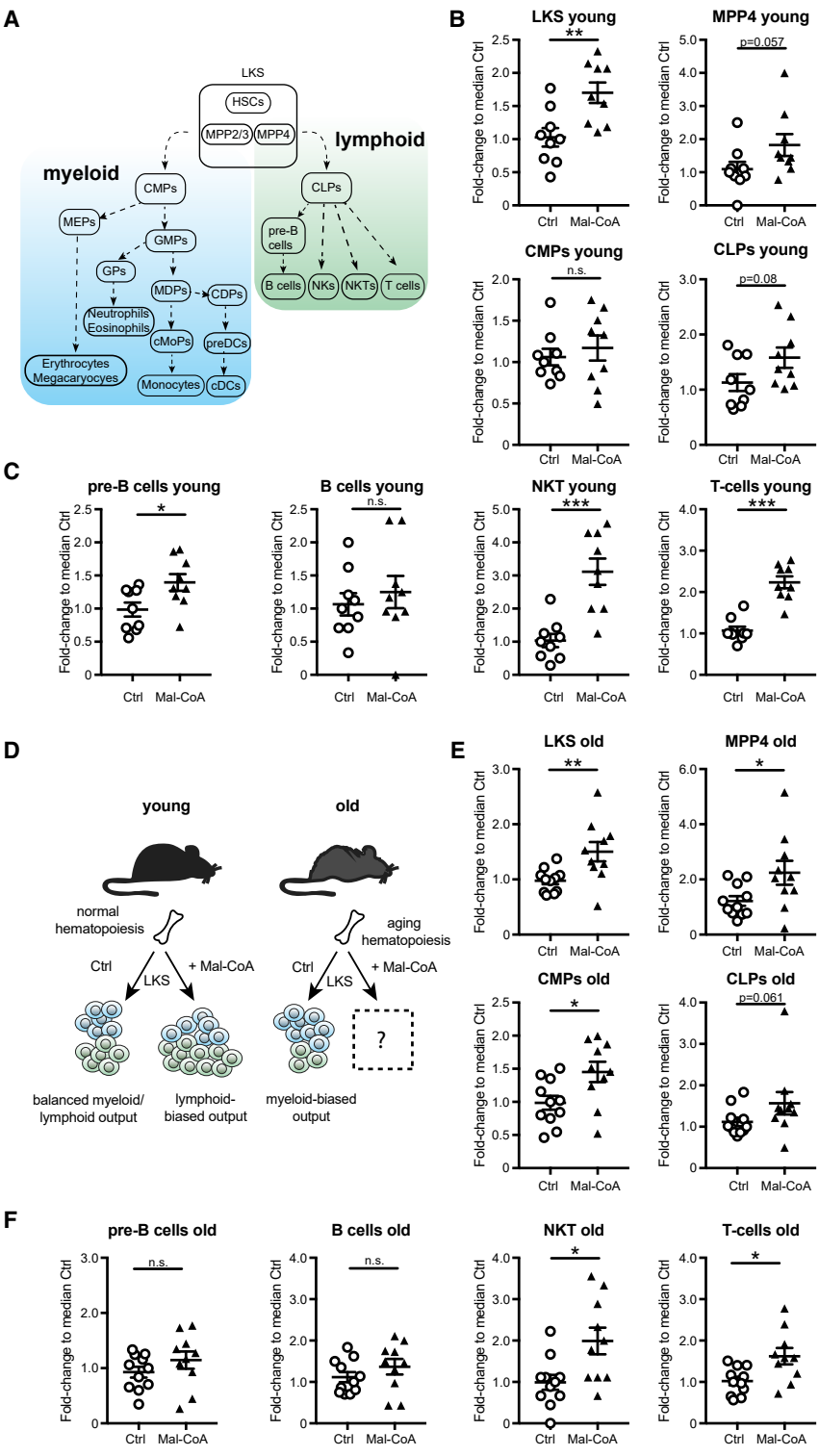

Figure 3. High-Dimensional Flow Cytometry Analysis Reveals Increased Lymphoid Populations Upon Malonyl-CoA Treatment

(A) Schematic overview of the lymphoid and myeloid hierarchies.

(B) Quantification by flow cytometry of the different progenitor populations, normalized to the median of the according control condition. This analysis revealed an increase in LKS cells upon culture with malonyl-CoA, as well as an increase in lymphoid progenitors ($p = 0.057$ for MPP4, $p = 0.08$ for CLPs), whereas myeloid-primed progenitors were not significantly altered.

(C) Analysis of differentiated lymphoid populations shows a significant increase in pre-B cells, NKT cells, and T cells. Statistical analysis was done including technical replicates using nonparametric Mann-Whitney test ($n = 9$, individual wells per condition, two independent experiments, cells isolated from 3 to 5 pooled mice respectively). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

(D) Schematic illustration of the changes in hematopoiesis between young and old mice.

(E) Quantification by flow cytometry of cultured LKS cells isolated from 2-year-old mice shows a significant increase in LKS cells upon culture with malonyl-CoA , as well as an increase in MPP4 progenitors and CLPs ($p = 0.061$). However, also myeloid-primed progenitors were significantly altered.

(F) Analysis of differentiated lymphoid populations show a significant increase in NKT and T cells after culture of old LKS cells with malonyl-CoA. Statistical analysis was done including technical replicates using nonparametric Mann-Whitney test (Ctrl $n = 11$, malonyl-CoA $n = 10$, individual wells, two independent experiments, cells isolated from 2 to 4 pooled mice respectively). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. The increase in LKS cells from old mice upon malonyl-CoA exposure was also observed in a third independent experiment with a less elaborate antibody panel, see Figure S4E.

significantly upon malonyl-CoA treatment (Figures 3E and S4E), suggesting that aged HSPCs remain indeed responsive. The lymphoid-biased shift in progenitor populations (MPP4 and CLPs) was also seen (Figures 3E and S4E). However, also CMPs were increased upon malonyl-CoA treatment (Figure 3E). The more differentiated lymphoid populations such as the pre-B cells and B cells showed a slight but nonsignificant increase with malonyl-CoA, while NKT and T-cells

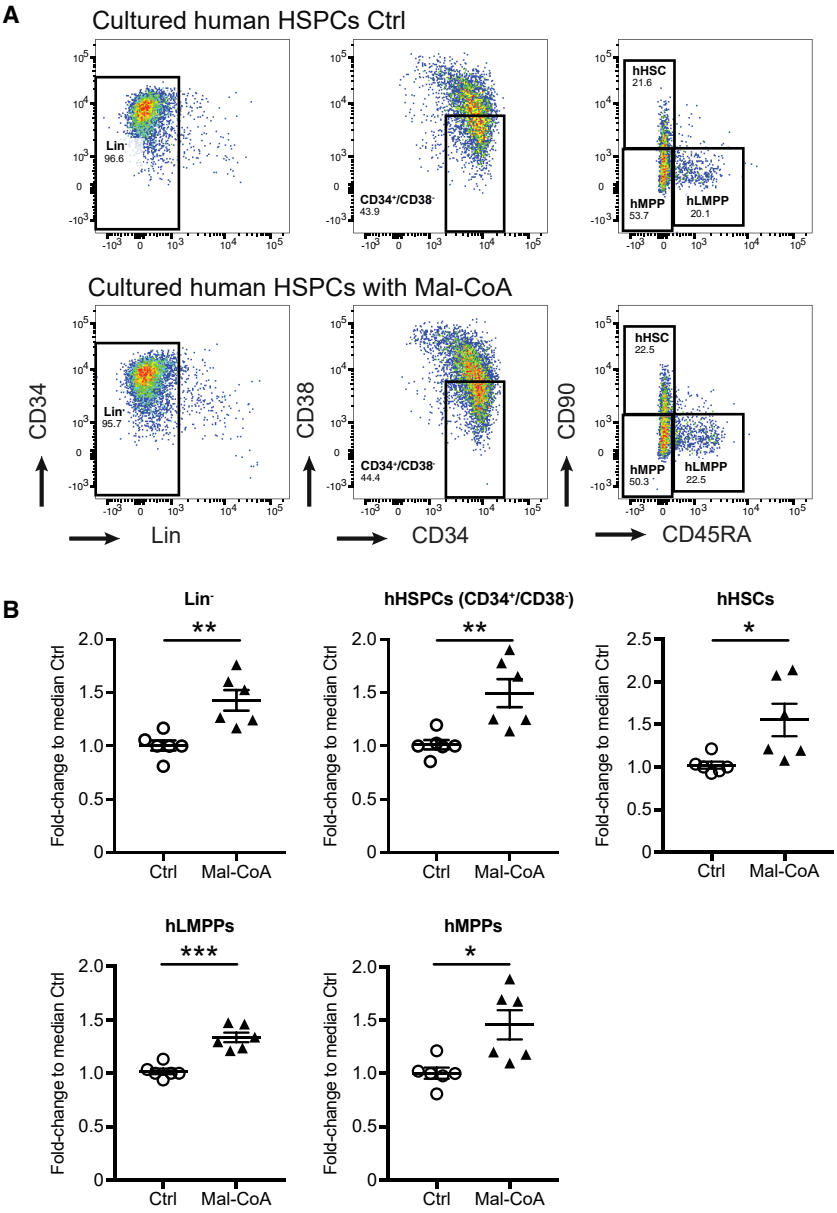

Figure 4. Malonyl-CoA Leads to Increased Proliferation of *In Vitro* Cultured Human HSPCs

(A) Surface marker expression of human HSPCs (Lin^-, $CD34^+$, $CD38^-$), cultured in the presence or absence of malonyl-CoA, was analyzed by flow cytometry. Representative flow cytometry plots showing the expression level of human $hLin^-$, $CD38^-/CD34^+$ (hHSPCs), lymphoid-primed multipotent progenitors (hLMPPs), multipotent progenitors (hMPPs) and hematopoietic stem cell (hHSC) markers.

(B) Quantification of the flow cytometry analysis. The total number of cells was normalized to the median of the control condition. Culturing of the Lin^- HSPCs in the presence of malonyl-CoA significantly expanded the number of total live cells as well as Lin^- cells. Further, malonyl-CoA treatment induced a significant increase in all the stem cell sub-populations (hHSPCs, hLMPPs, hMPPs and hHSCs). Statistical analysis was done including technical replicates by using nonparametric Mann-Whitney test (n = 6, individual wells per condition, two independent experiments, cells isolated from 2 × 2 pooled human cord blood samples). *p < 0.05; **p < 0.01; ***p < 0.001. The increase in hHSPCs, hHSCs, and hMPPs upon malonyl-CoA exposure was also observed in a third independent experiment with a similar experimental setup, see Figure S4F.

were significantly increased compared with controls, similar to young cells (Figure 3F). Committed myeloid progenitors did not change with malonyl-CoA (Figure S4D). Intriguingly, these data suggest that the lymphoid potential in aged mice can be increased with metabolic alteration and further corroborates the increase in the number of LKS cells upon malonyl-CoA exposure.

Malonyl-CoA Treatment Increases the Number of *In Vitro* Cultured Human HSPCs

Given the clinical relevance of HSPC transplantation in humans, together with the effects of malonyl-CoA on young and old mouse HSPCs *in vitro* and *in vivo*, we tested if malonyl-CoA affects human HSPCs. Human umbilical cord HSPCs (hHSPCs, $CD34^+/CD38^-$) were cultured for 5 days *in vitro* with or without the supplementation of malonyl-CoA. By flow cytometry, we found a significant increase in the percentage of $hLin^-$ cells as well as of all the human stem cell sub-populations ($CD34^+/CD38^-$ (HSPCs), lympho-myeloid progenitors (hLMPPs), hMPPs and hHSCs) after malonyl-CoA exposure (Figures 4A, 4B, and S4F), similar to the results obtained in mouse HSPCs. A titration curve with three different doses of malonyl-CoA further showed that this increase appears to be dose dependent (Figure S4G). Thus, a moderate modulation of lipid metabolism

through malonyl-CoA increases the number of human HSPC populations *in vitro* (Figure 4B).

DISCUSSION

The crucial role of metabolism in stem cell behavior in general (Chandel et al., 2016; Ito and Suda, 2014) and in HSPCs in particular (Ito and Ito, 2018) has been established over the past few years. The importance of glycolysis and mitochondrial activity for the most primitive HSPCs has led to various approaches to interfere with these metabolic pathways to promote HSPC expansion *in vitro*. The majority of those studies used chemicals to enhance glycolysis (Guo et al., 2018), block the fuel shuffling for oxidative phosphorylation (Takubo et al., 2013), or directly interfered with mitochondrial function, for instance by chemically uncoupling the electron transport chain (Vannini et al., 2016), or knocking out key mitochondrial players (Liu et al., 2015; Yu et al., 2013). These studies showed that manipulating metabolism is an approach to promote HSPC expansion. However, blocking key metabolic enzymes or mitochondrial function with chemicals might influence metabolism to a larger extent and for longer than aimed for. In this study, we exploited the modulation of lipid metabolism by increasing the levels of the endogenous metabolite malonyl-CoA, which acts as a block of Cpt1a (Foster, 2012) and as a building block for *de novo* lipogenesis. Our data show that by altering the levels of this single metabolite, HSPC behavior is influenced *in vitro* and *in vivo*. We found an approximately 3-fold increased number of HSPCs after culturing with malonyl-CoA. These findings are in line with data from Ito and colleagues (Ito et al., 2012), where an initial block of FAO with the irreversible drug etomoxir led to a short-term expansion of HSPCs. We show that the increased number of phenotypically defined HSPCs upon malonyl-CoA-induced *in vitro* expansion remained functional: when transplanted *in vivo*, malonyl-CoA–treated LKS cells showed a higher blood reconstitution potential than control LKS cells. These findings suggest that modulating the balance between FAO and *de novo* lipogenesis *in vitro* in LKS cells led to an increase of functional HSPCs and did not cause exhaustion of the stem cell pool, as seen with etomoxir. A possible explanation for these differences is that we have used a short-term, reversible modulation instead of an irreversible block, which might allow expansion of HSPCs without a change in stem cell potential. LKS cells exposed to malonyl-CoA even showed a slightly increased performance in a secondary transplant, however, this did not reach statistical significance.

Changes in gene expression were relatively small after malonyl-CoA exposure, indicating no radical change in cell identity, which is good when aiming for a clinical application to enhance HSPC performance. Our high-dimensional flow cytometry analysis suggests that malonyl-CoA specifically enhances lymphoid progenitors, corroborating the *in vivo* data showing increased lymphoid potential upon reconstitution. Whether this increase is due to a fate choice on the stem cell level or whether malonyl-CoA enhances the proliferation or survival of already established lymphoid progenitors remains to be determined.

Enhancing lymphoid reconstitution through metabolic modulation might be of clinical interest, as a rapid recovery of immune function is crucial for patients undergoing HSPC/BM transplantation (Copelan, 2006; Storek et al., 2008). Furthermore, HSPCs are biased toward myeloid cell production with aging, compromising immune system function (Kovtonyuk et al., 2016). Our data with HSPCs from old mice are very promising, showing that old HSPCs remain responsive to this metabolic modulation. Although the trends toward increased lymphoid-biased populations were the same as in young HSPCs, old HSPCs showed a greater variability upon malonyl-CoA exposure. Whether this reflects increased heterogeneity in the aged HSPC pool or whether the nonreactive HSPCs have irreversibly lost their lymphoid potential remains to be determined. In addition, myeloid progenitors (CMPs) were increased upon malonyl-CoA exposure in cells from aged mice, indicating that there might be a general proliferation or survival benefit.

Besides showing the power of metabolic modulation in mouse HSPCs, this study also corroborates the similarities in metabolism between different somatic stem cells. HSPCs and NSPCs reside in completely different locations and their progeny serve totally different functions; however, they are both increasing proliferation upon addition of malonyl-CoA into the culture medium (this study and Knobloch et al., 2017). As malonyl-CoA not only acts as a Cpt1 inhibitor, but also serves as a substrate for *de novo* lipogenesis (Foster, 2012), this activation might be the consequence of decreasing FAO and increasing *de novo* lipogenesis.

Strikingly, our data show that human cord blood HSPCs react in a similar way to the exposure of malonyl-CoA, opening an innovative avenue for treating cultured human HSPCs. Modulating lipid metabolism might be interesting in the context of therapeutic approaches where HSPCs have to be functionally maintained *in vitro*, such as gene therapy (Morgan et al., 2017). Whether malonyl-CoA has similar effects on human HSCPs derived from adult BM and if the age of the cells would influence the response to malonyl-CoA remains to be explored. In summary, we show that manipulation of a single metabolite, malonyl-CoA, is sufficient to alter mouse and human HSPC behavior and enhances lymphoid potential in young and old mouse HSPCs.

EXPERIMENTAL PROCEDURES

Cell Sources and Preparation

All *in vivo* procedures were carried out in compliance with the Swiss law after approval from the local authorities. C57Bl/6J (CD45.2) and B6.SJL-PtprcaPepcb/BoyCrl (Ly5.1 or CD45.1) were purchased from Charles River Laboratories International. Murine HSPCs were collected from freshly isolated BM. Human umbilical cord blood was obtained with parent written informed consent from cords/placentas of healthy full-term newborns.

Flow Cytometry

Mouse and human HSPCs were stained with fluorochrome-conjugated monoclonal antibodies listed under Supplemental Experimental Procedures. The cells were analyzed on the LSRII, LSR Fortessa (Becton Dickinson), or FACS Symphony A5 (Becton Dickinson/BD Biosciences). Cell sorting was performed by using FACS Aria II (Becton Dickinson) or MoFlo Astrios EQ (Beckman Coulter).

Cell Culture

Murine and human HSPCs were cultured with or without 5 to 200μM malonyl-CoA (CoALA Bioscience, MC01) for 5 to 7 days. A half medium change was performed after 3 or 4 days. Eighty murine HSCs (LKS $CD150^{+}CD48^{-}CD34^{-\ or\ +}$) were sorted into single wells of 96-round bottom microwell plates (VWR, 163320) or 10,000 murine HSPCs ($Lin^{-}Sca1^{+}cKit^{+}$) or human HSPCs ($Lin^{-}CD34^{+}CD38^{-}$) were seeded per well of a 48-well plate (Falcon, 353078). NSPCs were isolated and cultured as previously described (Knobloch et al., 2017).

RT-qPCR

A total of 25,000 LKS^{+}, w/o LKS^{+}, and Lin^{+} cells were sorted, RNA isolated with RNeasy Micro Kit (Qiagen, 74,004), followed by cDNA Synthesis (SuperScript IV, Invitrogen, 18091050). RT-qPCR was performed with Power SYBR-Green Master Mix (Thermo Fisher Scientific, 10658255). Primer sequences can be found under Supplemental Experimental Procedures.

RNA Sequencing

RNA from the progeny of 10,000 initially cultured LKS cells was extracted, purified, and checked for integrity. Cluster generation was performed and sequenced on the Illumina HiSeq 2500 using TruSeq SBS Kit v4 reagents. Purified and filtered reads were aligned against *Mus musculus.GRCm38.86* genome and differential expression was computed. The 123 differentially expressed genes (with an adjusted p value <0.1) were used for heatmap analysis using iDEP 0.90. web-tool.

FAO Measurements

FAO measurements were performed as previously described (Knobloch et al., 2017). Details can be found in the Supplemental Experimental Procedures.

Transplantation Assay

For the primary transplantation, nine recipient mice (CD45.2) per group were lethally irradiated 24 h before the i.v. injection of LKS cells (CD45.1, Donor, n = 10) cultured with or without 100μM Mal-CoA for 7 days and mixed with 350,000 freshly isolated competitor cells (CD45.1/2). For the secondary transplants, 5 Mio. of BM cells of each primary recipient was isolated 27 weeks post transplantation and injected in a 1:1 mode with freshly isolated competitor cells into lethally irradiated CD45.2 mice. Blood sampling and analysis of the recipient mice of the primary and secondary transplants were performed at 4, 8, 12, 16, 20 and 24 weeks posttransplant followed by final BM analysis.

Statistical Analysis

The following statistical tests were used: ordinary one-way ANOVA followed by Holm-Sidak's multiple comparison for RT-PCR data (dCt level); Mann-Whitney test for surface marker comparisons; two-way ANOVA (repeated measurement) followed by Holm-Sidak's for the transplantation data; and one-way ANOVA followed by Holm-Sidak's for titration curves. All statistical analyses were performed with Prism (GraphPad Software Inc). *In vitro* experiments were confirmed with at least two independent experiments. The key findings were confirmed in 3 to 6 independent experiments.

Data and Code Availability

Accession number for the RNA sequencing data:

The raw RNA sequencing data can be accessed via the GEO repository number GEO: GSE153201.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.stemcr.2020.07.021.

AUTHOR CONTRIBUTIONS

S.G. designed and performed experiments, analyzed and interpreted data, and co-wrote the manuscript. L.K. designed and performed human HSPC experiments and analyzed data. W.J.K performed FAO measurements. M.R., E.S., and V.I. performed the RNA sequencing analysis. S.U., L.K., and M.K. performed high-dimensional flow cytometry experiments. M.G., M.G.M., M.P.L., and S.J. provided key reagents, contributed to experimental design and helped with data interpretation. S.J. and M.K. developed the concept. M.K. designed and performed experiments, analyzed and interpreted data, and wrote the manuscript. All authors edited and reviewed the final manuscript.

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