Critical role of ASCT2-mediated amino acid metabolism in promoting leukaemia development and progression

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Amino acid metabolism is involved in diverse cellular functions, including cell survival and growth; however, it remains unclear how it regulates normal haematopoiesis versus leukaemogenesis. Here, we report that knockout of solute carrier family 1 member 5 (Slc1a5/ASCT2), a transporter of neutral amino acids, especially glutamine, results in mild-to-moderate defects in bone marrow and mature blood cell development under steady-state conditions. In contrast, constitutive or induced deletion of Slc1a5 decreases leukaemia initiation and maintenance driven by oncogene MLL-AF9 or phosphatase and tensin homologue (Pten) deficiency. Survival of leukaemic mice is prolonged following Slc1a5 deletion, and pharmacological inhibition of ASCT2 also decreases leukaemia development and progression in xenograft models of human acute myeloid leukaemia. Mechanistically, loss of ASCT2 generates a global effect on cellular metabolism, disrupts leucine (Leu) influx and mechanistic target of rapamycin (mTOR) signalling, and induces apoptosis in leukaemic cells. Given the substantial difference in reliance on ASCT2-mediated amino acid metabolism between normal and malignant blood cells, this in vivo study suggests ASCT2 as a promising therapeutic target for the treatment of leukaemia.

Cellular metabolism is remarkably changed in tumour cells as opposed to their normal counterparts. Tumour cells consume glucose at higher rates; however, in these highly proliferative cells, oxidation of glucose-derived pyruvate in the mitochondria is limited and a large portion of pyruvate is diverted to the cytosol for fermentation, even in the presence of ample oxygen. This aerobic glycolysis, known as the Warburg effect, is a hallmark of cancer cell metabolism1–4. The Warburg effect helps lower production of reactive oxygen species (ROS), the by-product of mitochondrial oxidative phosphorylation. In addition, decreased pyruvate oxidation in the mitochondria leads to elevated upstream glycolytic intermediates, which is beneficial for robust biosynthesis during tumour growth. Due to defective pyruvate oxidation in the mitochondria, cancer cells turn to alternative fuels, such as free fatty acids and amino acids to support oxidative phosphorylation15,16.

Amino acids represent an important class of major nutrients obligatory for cell survival and growth. They are not only used as building blocks for the synthesis of proteins, nucleotides and cellular primary antioxidant glutathione, but also play essential roles in energy production and intermediate metabolism in the mitochondria17. Intermediate metabolites produced by the tricarboxylic acid cycle in mitochondria are utilized for biosynthesis in the cytosol and participate in the epigenetic regulation of nuclear gene expression18,19. In addition, amino acids serve regulatory roles in governing cell growth, mainly through signalling to the energy, nutrient and growth factor integrating kinase mTOR20,21. Tumour cells have notably increased demands for these nutrients to support their exceptionally fast proliferation22. Essential amino acids must be obtained from external sources through transmembrane transporters. Non-essential amino acids can be synthesized endogenously, but also need to be obtained from external sources if the capacity of endogenous synthesis does not meet the increased demands of highly proliferative cells. ASCT2, also known as Slc1a5, is a transmembrane transporter that mediates the uptake of neutral amino acids, including glutamine (Gln), cysteine (Cys), serine (Ser), threonine (Thr), valine (Val) and alanine (Ala).23–25. ASCT2 is expressed at high levels in cancer, and knockdown or inhibition of ASCT2 has been shown to decrease cell growth in cell lines24–26. However, the role of ASCT2 in physiology has not been well characterized. Whether healthy cells and tumour cells of the same origin have differential responses to the loss of ASCT2 function in vivo settings remains to be determined.

The role of ASCT2-mediated amino acid metabolism in normal and malignant haematopoietic cell development is not well understood. We are interested in ASCT2 because our recent gene expression profiling analyses showed that Slc1a5 (ASCT2), along with other plasma membrane transporters and metabolic enzymes involved in amino acid metabolism, were significantly upregulated in mitochondrial phosphatidylinositol phosphate phosphatase PTPMT1 knockout haematopoietic stem cells (HSCs), wherein mitochondrial aerobic metabolism is decreased due to impaired utilization of pyruvate while cytosolic glycolysis is enhanced27,28. This apparently adaptive response of Slc1a5 expression in PTPMT1 knockout HSCs led us to determine the role of ASCT2-mediated amino acid metabolism in haematopoietic cell development. We found that deletion of ASCT2 had modest effects on steady-state...
normal blood cell development but substantially decreased leukaemia development and progression in mouse and xenograft models of human acute myeloid leukaemia (AML).

**Results**

**Deletion of Scl1a5 leads to mild defects in steady-state haematopoiesis.** Our recent quantitative reverse transcription PCR (qRT-PCR) analyses showed that levels of Scl1a5 (ASCT2) in HSCs were approximately sixfold higher than those in whole bone marrow (BM) cells (Supplementary Fig. 1a). Given that ASCT2 is responsible for the transport of neutral amino acids, especially Gln16, we examined the impact of Gln deprivation on HSCs in vitro. Compared to the control culture, myeloid (Mac-1+Gr-1+) cells derived from HSCs were drastically decreased in Gln-free medium, and approximately 90% of the cells remained at the lineage (Lin−) stage even after 14 d of culture (Supplementary Fig. 1b). The total cell number was approximately 20-fold lower than that of the control group (Supplementary Fig. 1c). To further determine the function of ASCT2 in haematopoietic cell development, we created an Scl1a5 conditional allele (Scl1a5Δ) and generated a global knockout allele (Scl1a5−/−) by crossing Scl1a5fl/fl mice with CMV-Cre mice to delete Scl1a5 from the germ line (Supplementary Fig. 2a,b). Depletion of ASCT2 at the messenger RNA (mRNA) and protein levels in homozgyous global knockout (Scl1a5−/−) mice was confirmed by qRT-PCR and western blot analyses (Supplementary Fig. 2c,d). Despite the profound impact of Gln deprivation on HSC differentiation in vitro (which may result from the combined effects of Gln starvation and oxidative stress during cell culture), surprisingly, Scl1a5−/− mice were born in a Mendelian ratio and had normal lifespans (Supplementary Fig. 3a). Total BM cellularity slightly decreased in Scl1a5−/− mice (Supplementary Fig. 3b). The frequencies of myeloid (Mac-1+Gr-1+), T lymphoid (CD3+), and B lymphoid (B220+) cells in the peripheral blood or BM were similar in Scl1a5−/− and wild-type (Scl1a5+/+) littermates, but no differences in red blood cells or platelets were observed between knockout and control animals (Supplementary Fig. 3a). Total BM cellularity slightly decreased in Scl1a5−/− mice (Supplementary Fig. 3b). The frequencies of myeloid (Mac-1+Gr-1+), T lymphoid (CD3+) and B lymphoid (B220+) cells in the peripheral blood or BM were similar in Scl1a5−/− and wild-type (Scl1a5+/+) mice (Supplementary Fig. 3c). Although a previous study showed that ASCT2 is required for erythroid specification in human CD34+ cells, we observed no effects of the loss of ASCT2 on erythroid cell development in steady-state haematopoiesis. Erythroid blasts in four differentiation stages developed without noticeable defects in Scl1a5 knockout mice (Supplementary Fig. 3d).

Scl1a5−/− mice had a marginal decrease in the frequencies and normal blood cell development but substantially decreased leukaemia development and progression in mouse and xenograft models of human acute myeloid leukaemia (AML).
these data suggest that ASCT2 is important for optimal HSC function in stress haematopoiesis but not for BM and mature blood cell development under steady-state conditions.

The role of ASCT2 in cellular metabolism is mediated by Gln and other amino acids. ASCT2 is a transporter for neutral amino acids and is thought of as a high-affinity Gln transporter. To determine whether the role of ASCT2 in HSCs was attributable to Gln transport, we examined Gln uptake in Slc1a5<sup>−/−</sup> and Slc1a5<sup>+/+</sup> Lin<sup>−</sup> cells and found that it was decreased by approximately threefold but not completely blocked in the knockout cells (Supplementary Fig. 6a).

We also assessed responses of Slc1a5<sup>−/−</sup> and Slc1a5<sup>+/+</sup> HSCs and LSK cells to low concentrations of Gln in in vitro culture. Slc1a5<sup>−/−</sup> cells were much more sensitive than their Slc1a5<sup>+/+</sup> counterparts to low Gln concentration–induced apoptosis (Supplementary Fig. 6b–d). Interestingly, even in the absence of Gln, Slc1a5<sup>−/−</sup> LSK cells still showed more apoptosis than Slc1a5<sup>+/+</sup> cells (Supplementary Fig. 6d), indicating that other amino acid substrates of ASCT2 may also mediate ASCT2 function.

Gln is an important carbon source and serves as an alternative fuel to support mitochondrial oxidative phosphorylation. We next assessed mitochondrial aerobic metabolism in intact, viable Cells in two femurs and tibias (×10<sup>4</sup>)

| LK | LSK | HSC |
|----|-----|-----|
| Slc1a5<sup>+/+</sup> | Slc1a5<sup>−/−</sup> |
| 0.00 0.01 0.02 0.03 0.04 | 0.1 0.2 0.3 0.4 0.5 |
| Percentage of BM cells | Percentage of BM cells |

| CLP | CMP | GMP | MEP |
|-----|-----|-----|-----|
| Slc1a5<sup>+/+</sup> | Slc1a5<sup>−/−</sup> |
| 0.00 0.01 0.02 0.03 0.04 | 0.1 0.2 0.3 0.4 0.5 |
| Percentage of BM cells | Percentage of BM cells |

| LSK cells | HSCs |
|-----------|-----|
| Slc1a5<sup>+/+</sup> | Slc1a5<sup>−/−</sup> |
| 0.00 0.01 0.02 0.03 0.04 | 0.1 0.2 0.3 0.4 0.5 |
| Apoptotic cells (%) | Apoptotic cells (%) |

| LSK cells | HSCs |
|-----------|-----|
| Slc1a5<sup>+/+</sup> | Slc1a5<sup>−/−</sup> |
| 0.00 0.01 0.02 0.03 0.04 | 0.1 0.2 0.3 0.4 0.5 |
| Apoptotic cells (%) | Apoptotic cells (%) |

| LSK cells | HSCs |
|-----------|-----|
| Slc1a5<sup>+/+</sup> | Slc1a5<sup>−/−</sup> |
| 0.00 0.01 0.02 0.03 0.04 | 0.1 0.2 0.3 0.4 0.5 |
| Apoptotic cells (%) | Apoptotic cells (%) |

| LSK cells | HSCs |
|-----------|-----|
| Slc1a5<sup>+/+</sup> | Slc1a5<sup>−/−</sup> |
| 0.00 0.01 0.02 0.03 0.04 | 0.1 0.2 0.3 0.4 0.5 |
| Apoptotic cells (%) | Apoptotic cells (%) |

| LSK cells | HSCs |
|-----------|-----|
| Slc1a5<sup>+/+</sup> | Slc1a5<sup>−/−</sup> |
| 0.00 0.01 0.02 0.03 0.04 | 0.1 0.2 0.3 0.4 0.5 |
| Apoptotic cells (%) | Apoptotic cells (%) |

| LSK cells | HSCs |
|-----------|-----|
| Slc1a5<sup>+/+</sup> | Slc1a5<sup>−/−</sup> |
| 0.00 0.01 0.02 0.03 0.04 | 0.1 0.2 0.3 0.4 0.5 |
| Apoptotic cells (%) | Apoptotic cells (%) |

| LSK cells | HSCs |
|-----------|-----|
| Slc1a5<sup>+/+</sup> | Slc1a5<sup>−/−</sup> |
| 0.00 0.01 0.02 0.03 0.04 | 0.1 0.2 0.3 0.4 0.5 |
| Apoptotic cells (%) | Apoptotic cells (%) |

| LSK cells | HSCs |
|-----------|-----|
| Slc1a5<sup>+/+</sup> | Slc1a5<sup>−/−</sup> |
| 0.00 0.01 0.02 0.03 0.04 | 0.1 0.2 0.3 0.4 0.5 |
| Apoptotic cells (%) | Apoptotic cells (%) |
Lin<sup>−</sup> cells by using real-time measurement of oxygen consumption. In the presence of Gln, Slc1a5 knockout cells showed lower basal oxygen consumption rates (OCR<sub>s</sub>) and maximal oxidative capacity compared to control cells (Supplementary Fig. 6e), suggesting decreased mitochondrial aerobic metabolism in Slc1a5 knockout cells. In the absence of Gln, the difference in mitochondrial maximal reserve capacities between Slc1a5<sup>−</sup> and Slc1a5<sup>+/+</sup> cells persisted, further supporting that loss of ASCT2 impacted cellular metabolism by decreasing the uptake of other neutral amino acids besides Gln. Measurement of extracellular proton flux showed that in the presence of Gln, Slc1a5-depleted cells had increased extracellular acidification rates (ECARs) (Supplementary Fig. 6f) indicative of enhanced glycolysis. Deprivation of Gln compensatorily enhanced glycolysis in Slc1a5<sup>−/−</sup> but not Slc1a5<sup>+/−</sup> cells (Supplementary Fig. 6f). Autophagy in Slc1a5<sup>−/−</sup> cells (Lin<sup>+</sup>) was possibly enhanced, as demonstrated by elevated levels of microtubule-associated proteins 1A/1B light chain 3A conjugated to phosphatidylethanolamine (LC3-II; Supplementary Fig. 6g). Steady-state total cellular ATP levels were lower in Slc1a5<sup>−/−</sup> cells compared to control cells (Supplementary Fig. 6h), and AMP-activated kinase, an intracellular energetic stress sensor, was activated in Slc1a5<sup>−/−</sup> knockout cells (Supplementary Fig. 6i). Consistent with these data, mTOR, the nutrient sensor and master regulator of protein translation, was inhibited in Slc1a5<sup>−/−</sup> cells, as evidenced by decreased phosphorylation of ribosomal protein S6 kinase 1 (S6K1) and S6 (Supplementary Fig. 6i).

Deletion of ASCT2 inhibits MLL-AF9-induced leukaemia. We next examined the role of ASCT2 in leukaemogenesis in vivo. Lin<sup>−</sup>BM cells isolated from Slc1a5<sup>−/−</sup> and Slc1a5<sup>+/+</sup> mice were transduced with the AML-associated oncogene MLL-AF9, and this was followed by inoculation into sublethally irradiated isogenic normal mice. All of the recipient mice (MLL-AF9; Slc1a5<sup>−/−</sup>) inoculated with MLL-AF9-transduced Slc1a5<sup>−/−</sup> cells died of AML within 45 d. In sharp contrast, survival of mice (MLL-AF9; Slc1a5<sup>−/−</sup>) inoculated with MLL-AF9-transduced Slc1a5<sup>−/−</sup> cells was prolonged by approximately 300 d (Fig. 2a). Proliferation of MLL-AF9-MLL-AF9<sup>−/−</sup> leukaemic cells was markedly lower compared to that of MLL-AF9-MLL-AF9<sup>−/−</sup> leukaemic cells in recipient mice (Fig. 2b). Leukaemic burden, as reflected by WBC counts (Fig. 2c), spleen weights (Fig. 2d) and the percentages of leukaemic cells (green fluorescent protein (GFP<sup>+</sup>)) in the peripheral blood, BM and spleen (Fig. 2e), was dramatically decreased in MLL-AF9; Slc1a5<sup>−/−</sup> recipients. Histopathological examination revealed that even 140 d after inoculation, AML phenotypes in the BM and spleen were much less severe, and infiltration of leukaemic cells in the liver and lung was much milder in MLL-AF9; Slc1a5<sup>−/−</sup> recipients than in MLL-AF9; Slc1a5<sup>−/−</sup> recipients at day 35 (Fig. 3f). Apoptosis of GFP<sup>+</sup> leukaemic cells, but not endogenous host leukocytes (GFP<sup>+</sup>), was doubled in MLL-AF9; Slc1a5<sup>−/−</sup> mice compared to that of MLL-AF9; Slc1a5<sup>−/−</sup> mice (Fig. 3g).

Deletion of ASCT2 suppresses Pten-loss-evoked blood malignancies. We next used a mouse genetics approach to further assess the role of ASCT2 in the development and progression of haematological malignancies. Pten<sup>−/−</sup>Mxl<sup>−/−</sup>; Slc1a5<sup>−/−</sup> and Pten<sup>−/−</sup>Mxl<sup>−/−</sup>; Slc1a5<sup>−/−</sup> mice were generated with Pten conditional and Slc1a5 global knockout mice. Even without pI:pC administration, Pten was partially deleted from these mice, due to the spontaneous activation of the Mxl promoter and Cre expression by endogenous basal interferon-α/β. Interestingly, Pten deletion efficiency in Pten<sup>−/−</sup>Mxl<sup>−/−</sup>; Slc1a5<sup>−/−</sup> mice was substantially lower than that in Pten<sup>−/−</sup>Mxl<sup>−/−</sup>; Slc1a5<sup>−/−</sup> mice (Supplementary Fig. 7a), indicating a growth disadvantage of Pten<sup>−/−</sup>Mxl<sup>−/−</sup>; Slc1a5<sup>−/−</sup> mice, consistent with previous studies<sup>23,24</sup>. These mice manifested elevated WBCs and increased myeloid cells (Mac-1<sup>+</sup>/Gr-1<sup>−</sup>) in the peripheral blood when they were 6–8 weeks old. However, no Pten<sup>−/−</sup>Mxl<sup>−/−</sup>; Slc1a5<sup>−/−</sup> mice showed MPN at this time point (Supplementary Fig. 7b,c).

Pten<sup>−/−</sup>Mxl<sup>−/−</sup>; Slc1a5<sup>−/−</sup> (Pten<sup>−/−</sup>; Slc1a5<sup>−/−</sup>−) and Pten<sup>−/−</sup>Mxl<sup>−/−</sup>; Slc1a5<sup>−/−</sup> (Pten<sup>−/−</sup>; Slc1a5<sup>−/−</sup>−) mice were then given pI:pC to further delete Pten<sup>−/−</sup>; Slc1a5<sup>−/−</sup>− mice developed severe MPN; two of the nine mice progressed into AML. They all died within 250 d of pI:pC administration. In sharp contrast, the survival of Pten<sup>−/−</sup>; Slc1a5<sup>−/−</sup>− mice was prolonged by >250 d (Fig. 4a). Although Pten<sup>−/−</sup>; Slc1a5<sup>−/−</sup>− mice subsequently developed MPN and died within 400 d of pI:pC administration, no acute leukaemia progression was detected. WBC counts (Fig. 4b), splenomegaly (Fig. 4c) and myeloid cells in the peripheral blood, spleen and BM were greatly decreased in Pten<sup>−/−</sup>; Slc1a5<sup>−/−</sup>− mice compared to Pten<sup>−/−</sup>; Slc1a5<sup>−/−</sup>+ control mice 45 d after pI:pC administration (Fig. 4d). Histopathological examination verified that leukaemic phenotypes in the BM and spleen were very attenuated; infiltration of neoplastic cells in the liver and lung was barely detectable in Pten<sup>−/−</sup>; Slc1a5<sup>−/−</sup>− mice at this time point (Fig. 4e). These data suggest that ASCT2 is essential for the initiation of haematological malignancies induced by loss of Pten.

To further determine the effect of ASCT2 deletion on the maintenance of established haematological malignancies induced by Pten deletion, we generated Pten<sup>−/−</sup>Mxl<sup>−/−</sup>; Slc1a5<sup>−/−</sup>− and Pten<sup>−/−</sup>Mxl<sup>−/−</sup>; Slc1a5<sup>−/−</sup>− mice with Pten and Slc1a5 conditional mice. As described earlier, due to the spontaneous activation of the Mxl promoter and Pten deletion (Supplementary Fig. 7d), Pten<sup>−/−</sup>Mxl<sup>−/−</sup>; Slc1a5<sup>−/−</sup>− control mice developed MPN when they were 6–8 weeks old. Pten<sup>−/−</sup>Mxl<sup>−/−</sup>; Slc1a5<sup>−/−</sup>− mice also displayed MPN phenotypes (Supplementary Fig. 7e–g). Interestingly, although Pten was similarly deleted (approximately 75%) in both types of
mice, *Slc1a5* was deleted from only approximately 40% of haematopoietic cells in *Pten<sup>fl/fl</sup>*; *Slc1a5<sup>−/−</sup>* mice (Supplementary Fig. 7d), further supporting the important role played by ASCT2 in the growth of *Pten*-deleted malignant cells.

*Pten<sup>fl/fl</sup>*; *Mx1-Cre; Slc1a5<sup>−/−</sup>* (*Pten<sup>−/−</sup>; *Slc1a5<sup>−/−</sup>*) and *Pten<sup>fl/fl</sup>*; *Mx1-Cre; *Slc1a5<sup>√/√</sup>* (*Pten<sup>−/−</sup>; *Slc1a5<sup>√/√</sup>*) diseased mice were then given pIpC to delete *Slc1a5* and further delete *Pten*. Three of the 12 *Pten<sup>−/−</sup>; *Slc1a5<sup>−/−</sup>* mice progressed to AML, whereas acute leukaemia progression was completely blocked in *Pten<sup>−/−</sup>; *Slc1a5<sup>√/√</sup>* mice. All *Pten<sup>−/−</sup>; *Slc1a5<sup>−/−</sup>* mice died with haematological malignancies within 50 d of pIpC administration. In contrast, the survival of double knockout (*Pten<sup>−/−</sup>; *Slc1a5<sup>−/−</sup>*) mice was significantly extended (Supplementary Fig. 8a). WBCs in the peripheral blood, splenomegaly and malignant cell infiltration in non-haematopoietic tissues were highly attenuated in *Pten<sup>−/−</sup>; *Slc1a5<sup>√/√</sup>* mice compared to *Pten<sup>−/−</sup>; *Slc1a5<sup>−/−</sup>* control littermates (Supplementary Fig. 8b–e). These data suggest that ASCT2 also plays an important role in the maintenance of haematological malignancies driven by *Pten* deletion.

To verify that the impact of ASCT2 deletion on MPN development and acute leukaemia progression is a cell-autonomous effect, we transplanted BM cells isolated from *Pten<sup>fl/fl</sup>*; *Mx1-Cre; *Slc1a5<sup>−/−</sup>* and *Pten<sup>fl/fl</sup>*; *Mx1-Cre; *Slc1a5<sup>√/√</sup>* mice (without pIpC administration) into lethally irradiated isogenic mice. Five weeks after transplantation, recipient mice were treated with pIpC to delete *Pten* (*Pten<sup>−/−</sup>; *Slc1a5<sup>−/−</sup>*) or both *Pten* and *Slc1a5* (*Pten<sup>−/−</sup>; *Slc1a5<sup>√/√</sup>*) mice.
from donor cells. All Pten+/−; Slc1a5+/− recipients developed MPN and progressed to acute leukaemias (AML and T cell acute lymphoblastic leukaemia). In contrast, only 40% of Pten+/−; Slc1a5+/− recipients showed acute leukaemia progression (60% of mice remained in MPN), and splenomegaly was highly attenuated in these mice (Supplementary Fig. 8f,g), reaffirming a critical cell-intrinsic role of ASCT2 in the development and progression of haematological malignancies.

**Loss of ASCT2 causes metabolic stress and apoptosis in leukaemic cells.** The mechanisms underlying the critical role of ASCT2 in leukaemic cells were investigated. Compared to Pten+/−; Slc1a5+/− Lin− cells, the Pten+/−; Slc1a5−/− counterparts showed decreased mitochondrial aerobic metabolism, as evidenced by reduced basal and maximal reserve OCRs (Fig. 5a,c). Interestingly, Pten+/−; Slc1a5−/− cells also displayed lower ECARs than Pten+/−; Slc1a5+/− cells (Fig. 5b,c). Reduction in both OCRs and ECARs in Pten+/−; Slc1a5−/− cells may be associated with increased apoptosis in these cells and indicates that Pten+/−; Slc1a5−/− cells become metabolically less active compared to highly energetic Pten+/−; Slc1a5+/− leukaemic cells. Ablation of ASCT2 in Pten+/−; Slc1a5−/− cells decreased ATP levels (Fig. 5d), although overall cellular ROS levels were not significantly changed (Fig. 5e) and autophagy was possibly enhanced in these cells, as demonstrated by elevated LC3-II levels (Fig. 5f). Loss of ASCT2 caused pronounced cell cycle arrest in the G0 phase; greatly increased apoptosis was detected in Pten+/−; Slc1a5−/− cells (Fig. 5g,h).

**ASCT2 deletion generates a global effect on leukaemia cell metabolism.** We next sought to determine the molecular mechanisms by which ASCT2 deletion impacts leukaemic cell metabolism. ASCT2 is known as a high-affinity transporter of Gln13, which participates in mitochondrial metabolism and synthesis of the cellular primary antioxidant glutathione, among other activities26,27. Gln uptake in ASCT2-depleted Pten+/−; Slc1a5−/− leukaemic cells (Lin−) was decreased by approximately 20-fold (Fig. 6a). Interestingly, the intermediate metabolites of the tricarboxylic acid cycle, α-ketoglutarate (α-KG) and acetyl-coenzyme A (CoA), were not significantly changed in Pten+/−; Slc1a5−/− cells (Fig. 6b,c), indicating that enhanced cell death in ASCT2-deleted leukaemic cells might not result from disruption of tricarboxylic acid cycle anaplerosis. The reduced (GSH) and oxidized (GSSG) glutathione ratio was decreased in these cells (Fig. 6d). Gln metabolism has an intricate interplay with glucose metabolism26,27; interestingly, despite the drastically decreased Gln uptake, glucose uptake in Pten+/−; Slc1a5−/− cells was lower than that in Pten+/−; Slc1a5+/− control cells (Fig. 6e); pyruvate, a key metabolite of glucose, was increased in Pten+/−; Slc1a5−/− cells (Fig. 6f).
Mass spectrometry-based comprehensive metabolomic profiling revealed that deletion of ASCT2 in Pten-deleted cells resulted in a global effect on cellular metabolism. Several metabolic pathways were severely impacted (Fig. 6g, Supplementary Fig. 9a, Supplementary Table 1). The glycolytic and pentose phosphate pathways were attenuated in Pten<sup>Δ/Δ</sup>; Slc1a5<sup>−/−</sup> cells, as demonstrated by a marked decrease in glucose 6-phosphate, ribulose 5-phosphate and nicotinamide adenine dinucleotide phosphate levels (Supplementary Fig. 9b). Surprisingly, Gln levels were not significantly changed in Pten<sup>Δ/Δ</sup>; Slc1a5<sup>−/−</sup> cells, possibly because it could be partially synthesized in the cells (Supplementary Fig. 9a,b). However, loss of ASCT2 decreased branched-chain amino acids in these cells (Supplementary Fig. 9c). In addition, levels of Cys and Thr were greatly decreased in ASCT2-depleted leukemic cells (Supplementary Fig. 9d). Despite these changes, purine and pyrimidine levels only marginally decreased (Supplementary Fig. 9e). Consistent with these massive metabolic changes, mTOR (both mTORC1 and mTORC2) signalling was inhibited, as demonstrated by substantially decreased phospho-mTOR, phospho-S6K1, phospho-S6 and phospho-Akt (S473) in Pten<sup>Δ/Δ</sup>; Slc1a5<sup>−/−</sup> cells (Fig. 6h). Cell survival and growth signaling, as reflected by phospho-Akt (T308) and phospho-Erk, was also inhibited, as demonstrated by substantially decreased phospho-mTOR, phospho-S6K1, phospho-S6 and phospho-Akt (S473) in Pten<sup>Δ/Δ</sup>; Slc1a5<sup>−/−</sup> cells (Fig. 6h).

Loss of ASCT2 inhibits Leu influx and mTOR signalling. To further define the mechanism underlying enhanced apoptosis in ASCT2-deleted leukemic cells, we performed rescue experiments in Pten<sup>Δ/Δ</sup>; Slc1a5<sup>−/−</sup> cells. Treatment with cell-permeable nucleosides, which can be phosphorylated in cells to produce nucleotides, the antioxidant N-acetyl-cysteine and the cell-permeable α-KG analogue dimethyl-α-KG, which should fulfill many of the bioenergetic roles of Gln, failed to rescue Pten<sup>Δ/Δ</sup>; Slc1a5<sup>−/−</sup> cells (Fig. 7a–c). Given that Gln uptake was dramatically decreased and that mTOR signalling was diminished in Pten<sup>Δ/Δ</sup>; Slc1a5<sup>−/−</sup> cells, we reasoned that Leu influx, which relies on Gln efflux to activate mTOR<sup>33</sup>, might be reduced in these cells. Indeed, Leu uptake was decreased by half in Pten<sup>Δ/Δ</sup>; Slc1a5<sup>−/−</sup> cells compared to Pten<sup>Δ/Δ</sup>; Slc1a5<sup>+/+</sup> cells (Fig. 7d). Treatment with the cell-permeable Leu analogue l-leucyl-l-leucine methyl ester (LLME)<sup>29</sup>, which does not require cell transporters to enter cells, reversed the increased apoptosis in Pten<sup>Δ/Δ</sup>; Slc1a5<sup>−/−</sup> cells (Fig. 7e). LLME treatment mitigated
Fig. 5 | Deletion of ASCT2 decreases mitochondrial metabolism, and induces cell cycle arrest and apoptosis in Pten-deficient leukaemic cells.

- Cell cycle arrest and senescence in these cells (Fig. 7g). Treatment with the mTOR activator MHY1485 also effectively improved survival in Pten+/Slc1a5−/− cells (Fig. 7h). Importantly, LLME largely corrected mTOR signalling in Pten+/Slc1a5−/− cells (Fig. 7i), suggesting that the enhanced leukaemic cell death induced by ASCT2 deletion was attributable to subsequently decreased Leu uptake and diminished mTOR activity.

Furthermore, to verify the role of decreased Leu uptake in the increased apoptosis of Slc1a5−deleted leukaemic cells in vivo, we treated MLL-AF9; Slc1a5−/− and Pten+/Slc1a5−/− leukaemic mice with LLME, which showed substantially prolonged survival compared to MLL-AF9; Slc1a5+/+ and Pten+/Slc1a5+/+ leukaemic mice (Figs. 2a and 4a). LLME treatment markedly shortened their survival due to increased leukaemic burden and progression, whereas MLL-AF9; Slc1a5+/+ mice were not affected by LLME treatment (Fig. 7j–o). These in vitro and in vivo rescue data collectively suggest that the enhanced leukaemic cell death induced by ASCT2 loss was mainly attributed to subsequently decreased Leu influx and inhibition of mTOR.

Likewise, Leu uptake decreased in Slc1a5−deleted normal cells (Lin−) compared to the undeleted counterparts (Supplementary Fig. 10a). Slc1a5−/− HSCs and LSK cells cultured in a low concentration of Gln were partially rescued by treatment with LLME or MHY1485, and mTOR signalling was largely corrected by LLME and MHY1485 (Supplementary Fig. 10b–e). Interestingly, Slc1a5−deleted normal cells also showed partial rescue responses to dimethyl-α-KG (Supplementary Fig. 10b–e), in contrast to Slc1a5−deleted leukaemic cells (Fig. 7c). Together, these data indicate that both reduced mitochondrial oxidative phosphorylation and diminished mTOR signalling caused by ASCT2 deficiency...
Fig. 6 | Loss of ASCT2 generates a global effect on cellular metabolism in Pten-deficient leukaemic cells. a-f, Lin− cells were isolated from 6- to 8-week-old Pten+/+Mx1-Cre; Slc1a5−/− (Pten+/+; Slc1a5−/−) and Pten+/+Mx1-Cre (Pten+/+; Slc1a5+/−) mice 10 d after pCp administration. Cells were processed for Gln uptake as described in the Methods (a, n = 3 mice per genotype). Data are presented as the mean ± s.d. of biological replicates. ***P < 0.001 (unpaired, two-tailed Student’s t-test). Intracellular α-KG (b, n = 5 mice per genotype), acetyl-CoA (c, n = 5 mice per genotype), GSH/GSSG ratio (d, n = 3 mice per genotype) and pyruvate (f, n = 5 mice per genotype) were determined as described in the Methods. These cells were also incubated with 2-N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose and analysed for glucose uptake using FACS (e, n = 3 mice per genotype). Data are presented as the mean ± s.d. of biological replicates. *P < 0.05; **P < 0.01; ***P < 0.001 (unpaired, two-tailed Student’s t-test). g, h, i. Whole-cell lysates prepared from Lin− cells were examined by immunoblotting with antibodies to phospho-mTOR, phospho-S6K1, phospho-S6, phospho-Akt, phospho-Erk, Pten and ASCT2. Densitometric data of phosphoproteins (normalized to pan proteins) from three independent experiments are shown on the right (h). Data are presented as the mean ± s.d. of biological replicates. **P < 0.01; ***P < 0.001 (unpaired, two-tailed Student’s t-test). j. BM sections prepared from Pten+/+; Slc1a5−/− and Pten+/+; Slc1a5+/− mice (n = 4 mice per genotype) were examined using immunohistochemical staining for phospho-S6K1. Representative images from four independent experiments are shown.

contributed to the increased apoptosis in Slc1a5-deleted normal cells, whereas in Slc1a5-deleted leukaemic cells, defective mTOR signalling, but not mitochondrial metabolic defects, was responsible for increased cell death.

Slc1a5-deleted leukaemic cells (Pten+/+; Slc1a5−/−) and Slc1a5-undeleted control leukaemic cells (Pten+/+; Slc1a5+/−) showed similar sensitivity to further Gln deprivation (Supplementary Fig. 10fg), although Pten+/+; Slc1a5−/− cells exhibited elevated apoptosis in the presence of saturated Gln (2 mM). Treatment with cell-permeable analogues of Gln, Leu, Cys, Val or Arg, but not Lys, partially reversed the increased apoptosis in Pten+/+; Slc1a5−/− cells. Notably, among the amino acids tested, LLME showed the best rescue effect (Supplementary Fig. 10h). These data suggest that the enhanced cell death caused by ASC2 depletion in leukaemic cells was mainly caused by greatly decreased Gln uptake and subsequent defective Leu influx; yet, reduced transport of other substrate amino acids also contributed to the decreased survival of Slc1a5 knockout leukaemic cells. In addition, to determine whether LLME acted as a bona fide Leu donor, we deprived Pten+/+; Slc1a5+/− leukaemic cells of Leu in the presence or absence of LLME. Leu deprivation induced apoptosis in Pten+/+; Slc1a5+/− cells, mimicking the phenotype of Pten+/+; Slc1a5−/− cells. LLME treatment reversed apoptosis in Pten+/+; Slc1a5+/− cells deprived of Leu, whereas it did not affect the survival of unstarved Pten+/+; Slc1a5+/− cells (Supplementary Fig. 10i).

Inhibition of ASC2 suppresses human AML in xenograft models. Given the significant difference in responses to Slc1a5 (ASC2) deletion between normal haematopoietic cells (except stress conditions) and malignant cells, and the remarkable effects of ASC2 deletion on leukaemia initiation and maintenance in mouse leukaemias, we tested for the potential therapeutic effect of pharmacological blockade of ASC2 in human AML. The treatment of patient primary AML cells with l-c-glutamyl-p-nitroanilide (GPNA), an inhibitor of ASC2 (ref. 30), decreased cell survival (Fig. 8a) and increased apoptosis in vitro (Fig. 8b). However, no significant
effects on cell survival were observed in normal BM cells (Fig. 8a,b). Functional colony assays verified that GPNA markedly decreased the ability of AML cells, but not normal BM progenitors, to form colonies (Supplementary Fig. 11a), which is indicative of a potential therapeutic index of ASCT2 inhibition.

We next set up xenograft models of human AML in NOD/scid IL2Rgamma mice. When human CD45+ AML cells became detectable, xenografted mice were treated with GPNA or vehicle for 4 weeks. GPNA treatment effectively suppressed leukaemia progression. Splenomegaly and hepatomegaly were attenuated (Fig. 8c,d). The WBCs in the peripheral blood of GPNA-treated leukaemic mice decreased (Supplementary Fig. 11b). Furthermore, patient CD34+ AML cells drastically decreased in the peripheral blood and BM following treatment with GPNA (Fig. 8e,f). Compared to vehicle-treated mice, GPNA-treated leukaemic mice showed greatly increased apoptosis in human leukaemic cells in both peripheral blood and BM (Fig. 8e,f). Pathological examination verified that leukaemic phenotypes in the BM and spleen were attenuated, and infiltration of leukaemic cells into non-haematopoietic organs was much reduced in GPNA-treated mice (Supplementary Fig. 11c). These preclinical data validate the critical role of ASCT2 in human leukaemia development and progression. This notion is further supported by the correlation of Slc1a5 amplification or high expression with poor prognosis in human AML patients (Supplementary Fig. 11d); mutations in four AML-associated genes (TP53, XIAP, PHH6 and GRIK4) have a tendency to significantly co-occur with Slc1a5 amplification (Supplementary Fig. 11e).

Discussion

Although several amino acids have been shown to be important for cancer cell metabolism and growth, little progress has been made in finding therapeutic targets (except for l-asparaginase) in amino acid metabolic pathways that can be harnessed to kill cancer cells but spare normal cells. In this study, we demonstrated in in vivo settings that normal and malignant haematopoietic cells had remarkably differential responses to blockade of ASCT2-mediated amino acid metabolism. Slc1a5 (ASCT2) knockout mice had normal lifespans without noticeable abnormalities. This amino acid transporter was not critically important for mature blood cell production in steady-state haematopoiesis, although it was required for optimal HSC regenerative capabilities under stress conditions. ASCT2 deletion showed only mild-to-moderate effects on blood cell development, consistent with recent findings that ASCT2 is dispensable for homeostatic T cell development, although it was important for T cell activation1, and that no abnormalities were found in B cell development and function in the absence of ASCT2 (ref. 33). In contrast, ASCT2 was much more important for leukaemia development and progression. Constitutive or induced deletion of ASCT2 greatly decreased initiation and maintenance of haematological malignancies driven by MLL-AF9 or Pten deficiency. Survival of ASCT2-deleted leukaemic mice was prolonged by 200–300 d. Furthermore, pharmacological inhibition of ASCT2 produced a significant therapeutic effect in xenograft models of human AML, while the impact on normal blood cell formation was minimal.

ASCT2 appears to play a pleiotropic role in cell metabolism; enhanced cell death in leukaemic cells induced by ASCT2 deletion was probably attributed to multiple metabolic defects. Although ASCT2 is known as a high-affinity Gln transporter3, and Gln uptake was indeed drastically decreased in Slc1a5 knockout leukaemic cells, deletion of ASCT2 produced a pronounced global effect on cell metabolism, which cannot be fully explained by its function in transporting Gln. In fact, Gln and glutamate levels were only slightly but not significantly reduced in ASCT2-deleted leukaemic cells, possibly because Gln could also be transported through Slc38a5, Slc38a1 or Slc38a2, and be partially synthesized in cells. Other amino acid substrates beyond Gln might also mediate the function of ASCT2. Levels of Cys, Val, Ile and Thr were significantly decreased in Slc1a5 knockout leukaemic cells. These amino acids are critical for the maintenance of intracellular redox homeostasis and robust biosynthesis in cancer cells34-36. Conceivably, reduction in the transport of these amino acids also contributed to the enhanced cell death in ASCT2-deleted leukaemic cells. This notion is supported by the partial rescue effects of cell-permeable analogues of Cys and Val on the survival of Slc1a5-deleted leukaemic cells. Moreover, many other metabolic pathways, such as the glycolytic and pentose phosphate pathways, and the methylation cycle, were diminished in ASCT2-deleted leukaemic cells. These broad metabolic changes might account for the profound impact of ASCT2 deletion/inhibition on leukaemic cells and the more efficacious effects of targeting ASCT2 over a single enzyme involved in the Gln metabolic pathway in malignant cells.

In addition, functional coupling of ASCT2 with other amino acid transporter(s) is critical for leukaemic cell metabolism. The...
influx of Leu and other amino acids requires simultaneous efflux of Gln and its derivatives (for example, glutamate) through the large neutral amino acid transporter (Slc7a5)\textsuperscript{28,35} and other bidirectional transporter(s), respectively, although in some cell lines Leu import does not rely on ASCT2 (ref. \textsuperscript{36}). Although steady-state levels of Gln in ASCT2-deleted leukaemic cells were slightly reduced, Gln uptake was decreased by approximately 20-fold in these cells, which could lead to reduced Gln efflux rates and thus Leu influx. Decreased Leu uptake in turn resulted in diminished mTOR signalling in ASCT2-deleted leukaemic cells, since a constant intracellular pool of Leu is required for the activation of mTORC1 (refs. \textsuperscript{28,35}). Indeed, treatment with a cell-permeable Leu analogue (LLME) not only largely restored mTOR signalling but also rescued survival in ASCT2-deleted cells in vitro and in vivo. Therefore, it is likely that reduced
uptake of Gln and other neutral amino acids, combined with the functional uncoupling of ASCT2 and the large neutral amino acid transporter, and possibly other bidirectional transporters, collectively led to the pleiotropic metabolic effects of ASCT2 deletion/inhibition in leukaemic cells.

Another interesting finding is that normal haematopoietic cells and leukaemic cells had substantially differential reliance on ASCT2. The effects of ASCT2 deletion on both mitochondrial oxidative phosphorylation and mTOR activation were associated with decreased survival in normal haematopoietic stem/progenitor cells; the cell-permeable α-KG analogue and the mTOR activator rescued Slc1a5 knockout cells. However, mitochondrial metabolic defects caused by ASCT2 deletion did not contribute significantly to enhanced apoptosis in Slc1a5 knockout malignant cells because α-KG failed to rescue Slc1a5-deleted leukaemic cells, possibly due to unique rewired/reprogrammed metabolic processes in malignant cells. Rather, diminished mTOR signalling was probably responsible for the increased apoptosis in Slc1a5-deleted leukaemic cells, given that LLME (and also the mTOR activator MHY1485) effectively restored mTOR signalling and rescued survival in these cells. Transformed cells were much more sensitive to ASCT2 deletion than steady-state normal stem/progenitor cells. This might be partly due to the rapid proliferation and robust biosynthesis and/or reprogrammed cellular metabolism in tumour cells. However,
the detailed mechanisms underlying the differential responses to ASCT2 deletion/inhibition between normal and malignant haematopoietic cells remain to be determined.

Finally, our study suggests that ASCT2 holds great promise as an effective molecular target for the treatment of leukaemia. The ASCT2 inhibitor GPNA showed a significant therapeutic effect in the xenograft models of human AML in this study; however, the potency of GPNA is low and it is not specific for ASCT2. It also inhibits sodium-independent carriers.51,52 A new competitive, selective and potent ASCT2 antagonist (V-9302) has recently been developed53. V-9302 inhibited the uptake of Glu and other neutral amino acids with much improved potency. This antagonist produced a marked therapeutic effect in nude mice xenografted with colon cancer cell lines55. Nevertheless, although our overall findings strongly suggest ASCT2 as a therapeutic target for leukaemia treatment, caution should be exercised in clinical trials when considering combined treatments with ASCT2 inhibition and DNA-damaging chemotherapy, which impose tremendous stress on HSCs. Given that ASCT2 is required for optimal HSC regenerative capabilities under stress conditions, combined treatments might cause severe adverse effects on normal haematopoiesis in patients.

Methods

Mice and patient samples. Slc1a5+ mice (in the C57BL/6 genetic background) were generated using a conventional gene-targeting strategy, wherein exons 2–4 were flanked by two loxP sites, through homologous recombination. CMV-Cre (stock no. 006054), Mxi1-Cre (stock no. 003556) and Ptenfl/+ (stock no. 006440) mice in the C57BL/6 background were purchased from the Jackson Laboratory. Slc1a5+ mice were crossed with CMV-Cre transgenic mice to delete Slc1a5 from the germ line to generate global heterozygous knockouts (Slc1a5fl/+ mice). Slc1a5fl/+ mice were also crossed with Mxi1-Cre mice to generate inducible haematopoietic cell knockouts (Slc1a5flMxi1-Cre mice). Mice of the same age (specified in the figure legends), sex and genotype (often from the same litters, but not always) were mixed and then randomly grouped for subsequent analyses. (Investigators were not blinded.) Mice were kept under specific-pathogen-free conditions at Emory University, Division of Animal Resources. All animal procedures complied with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Experiments involving leukaemia induction in mice were approved by the Institutional Animal Care and Use Committee (IACUC). Mice were monitored daily for any sign of discomfort and pain by our laboratory personnel as well as the staff at the Division of Animal Resources facility. In addition to monitoring the pathological status of the mice, we followed IACUC guidelines for end points. Any animals showing signs of discomfort, pain or distress listed in IACUC guidelines and recommended for euthanization by the animal resources facility staff were killed. De-identified BM biopsies from AML patients without any patient information were obtained from Emory University Hospital. The experiments involving human participants were reviewed and approved (exemption IV) by the institutional review board of Emory University.

Flow cytometry analysis. To isolate Lin− cells, BM cells were lineage-depleted using a lineage depletion kit (Miltenyi Biotech). The pool size, cell cycle status and apoptosis of HSCs and LSK cells were analysed by multi-parameter fluorescence activated cell sorting (FACS) analyses, as previously described40. In brief, BM cells freshly collected from femurs and tibias were stained with antibodies labelled with various fluorochromes (eBioscience and BD Biosciences), stained with Ki-67 antibody and further incubated with Hoechst 33342 (20 μg/ml). For the apoptosis analyses, fresh BM cells were stained for HSC markers as stated earlier, fixed and permeabilized using a Cytofix/Cytoperm kit (BD Biosciences), stained with Ki-67 antibody and further incubated with Hoechst 33342 (20 μg/ml). For the apoptosis analyses, fresh BM cells were stained for HSCs and then incubated with annexin V and 7-aminominoacycin D (BD Biosciences). To measure cellular ROS levels, cells were incubated in PBS containing 2−7−dichlorofluorescein diacetate (5 μM) at 37 °C for 15 min. Cells were then washed with PBS. ROS (H2O2) levels were quantified using FACS analyses. To measure glucose uptake, cells were incubated with 2-((N-(7-nitro-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (200 μM) at 37 °C for 30 min. Cells were washed with PBS and then analysed with FACS. To measure senescent cells, cells were incubated with 5-dodecanoylaminofluorescein diacetate (33 μM) for 1 h, followed by FACS analyses. Data were collected on a BD LSRII Flow Cytometer (BD Biosciences) and analysed with the FlowJo software (TreeStar). The cell gating strategies used in the FACS analyses and cell sorting are shown in Supplementary Fig. 12.

CFU assay. Freshly collected mouse BM cells (2×106 cells/ml−1) were plated in triplicate in 35-mm dishes in 0.9% methylcellulose Iscove’s modified Dulbecco’s medium (IMDM) containing 15% foetal bovine serum (FBS), Glu (10 mM), β-martcopoethanol (3.3×10−3 M), stem cell factor (50 ng/ml−1), interleukin-3 (1×10−2), IL-3 (20 ng/ml−1), IL-6 (20 ng/ml−1) and erythropoietin (3 units/ml−1). After 10 d of incubation at 37 °C in 5% CO2, haematopoietic colonies (CFU-GEMM, CFU-GM and burst-forming unit-erythroid cells) were counted under an inverted microscope.

For human marrow cell CFU assays, marrow biopsies from AML patients or normal individuals (1×106 cells/ml−1) were plated in triplicates in 35-mm dishes in 0.9% methylcellulose IMDM medium containing 15% FBS, human stem cell factor (50 ng/ml−1), human IL-3 (10 ng/ml−1), human IL-6 (10 ng/ml−1), human granulocyte-macrophage colony-stimulating factor (25 ng/ml−1) and erythropoietin (3 Units/ml−1). GPNA (1 mM; Sigma-Aldrich) or vehicle was added to the culture medium. Cells were incubated at 37 °C in 5% CO2 for 10 d. Colonies were scored with an inverted microscope.

Competitive repopulation assay. Total test BM cells (2×106) freshly collected from Slc1a5+ and Slc1a5− mice (CD45.2+) were transplanted with the same number of BM cells (competitor cells) isolated from BoyJ mice (CD45.1+) into lethally irradiated (1,100 rad) BoyJ recipients through tail vein injection. Test cell reconstitution (CD45.2+) in the whole cell population was determined at 4, 8, 12 and 16 weeks after transplantation using FACS analyses of peripheral blood and BM cells as previously described10,11. BM cells collected from recipients 16 weeks after primary transplantation were transplanted (1×106 cells per mouse) into lethally irradiated secondary BoyJ recipients. Test cell reconstitution in the peripheral blood and BM cells was determined using FACS 4, 8, 12 and 16 weeks after secondary transplantation as stated earlier.

Retroviral BM cell transduction. For virus packaging, retroviral constructs MSCV-MLL-AF9-IRES-GFP were co-transfected with pEJ-Pam3 (E) and pSRαG packaging vectors into HEK293T cells by using calcium phosphate-mediated transfection. Virus-containing supernatant was collected 48–72 h post-transfection and used for infection. In brief, Lin− cells isolated from the BM were incubated in IMDM supplemented with stem cell factor (50 ng/ml−1), thrombopoietin (50 ng/ml−1), IL-3 (10 ng/ml−1) and IL-6 (10 ng/ml−1) (all from PeproTech) for 24 h. Cells were then spin-injected with retroviral supernatant supplemented with polybrene (8 ng/ml−1) at 490g for 45 min at 20 °C. Infected cells were transplanted into sublethally irradiated (700 rad) BoyJ mice (2×106 cells per mouse) by tail vein injection for in vivo expansion of leukaemic cells for 6 weeks. GFP+ cells were sorted from the BM of these mice and then transplanted into sublethally irradiated new batches of BoyJ mice (1×106 cells per mouse).

Diagnosis of MPN and AML in mice. Diagnosis of genetically modified mice or leukaemic cell transplants was made as described in previous reports from our laboratory and others48,54,55. Specific criteria for MPN, AML or T cell acute lymphoblastic leukaemia are listed in Supplementary Table 2.

Measurement of oxygen consumption. Measurement of intact cellular respiration was performed using the Seahorse XF480 Metabolic Flux Analyzer (Seahorse Biosciences). In brief, Lin− cells were seeded onto Cell-Tak pre-coated XPl cell culture miniplates and incubated in Seahorse XF Base Medium with 25 mM glucose at 37 °C for 1 h. Respiration was analysed in XF Base Medium (containing 25 mM glucose) with or without 10 mM Glu, and in the presence of the mitochondrial inhibitor oligomycin (50 nM), mitochondrial uncoupling compound carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP) (5 μM) and respiratory chain inhibitor rotenone (1 μM). All measurements were performed following the manufacturer’s instructions and protocols. OCRs (pmol min−1) and ECARs (μM HEPES pH 7.4) were determined.

Intracellular metabolite measurements. The intracellular levels of pyruvate, α-KG and acetyl-CoA were determined using commercial kits (BioVision). In brief, 2×106 cells were homogenized in PBS. The supernatant was collected and the proteins were removed by using 10kDa Amicon Ultra Centrifugal Filters (Millipore). The flow-through containing the metabolites was used for the measurement of acetyl pyruvate, α-KG and acetyl-CoA, following the manufacturer’s instructions. The intracellular GSH/GSSG ratio was determined using GSH/GSSG-Glo (Promega), following the manufacturer’s instructions. Total cellular ATP levels in live cells were measured using an ATP assay kit (Sigma-Aldrich).

Measurement of 14C-labelled Glu and Leu uptake. Cells were starved in Glu-free or Leu-free Roswell Park Memorial Institute medium for 4 h, and 14C-Glu (10 mM; Toronto Research Chemicals) or 14C-Leu (10 mM; Cambridge Isotope Laboratories) was then added to the medium. After 10 min of incubation, cells were washed twice with cold PBS and 5 ml methanol, snap-frozen three times and centrifuged (3,000g, 30 min) to remove cell debris. After sample normalization based on total protein levels, α-KG levels or 14C-labelled Glu and Leu uptake were quantified using liquid chromatography–tandem mass spectrometry.

Metabolomic analysis. Six to eight-week-old Pten−/−, Slc1a5+/− and Pten−/−, Slc1a5−/− mice were administered three doses of ip[13C] (1.0μg/g body weight) every other day over 5 d to delete the Pten gene. Ten days later, Lin− cells were
freshly isolated from the BM for metabolome analyses. In brief, cells were washed in 5% mannitol and then plunged into methanol that contained internal standard solutions (Human Metabolome Technologies America) for cations and anions. The supernatants were extracted and prepared for analyses according to the protocol provided by Human Metabolome Technologies America. The extracted samples were further prepared for metabolome analyses using an Agilent capillary electrophoresis time-of-flight mass spectrometry system.

Statistics and reproducibility. Unless otherwise noted, data are presented as the mean ± s.d. of biological replicates (independent animals/independent experiments; the n numbers are specified in each figure legend). An unpaired, two-tailed Student's t-test was used for the statistical comparison of two groups. For the Kaplan–Meier survival analysis, the log-rank (Mantel–Cox) test was used to determine statistical significance. *P < 0.05; **P < 0.01; ***P < 0.001.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request. The Reporting Summary for this article is available as a Supplementary Information file.

Received: 13 June 2018; Accepted: 28 January 2019; Published online: 11 March 2019

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Acknowledgements
This work was supported by the National Institutes of Health grant nos. DK092722 and HL110995 (to C.K.Q.).

Author contributions
F.N. and W.M.Y. generated and characterized the haematopoietic cell development in Slat1A5 global and conditional knockout mice, set up the mouse leukaemia models and xenograft models of human AML and analysed leukaemia development/progression. F.N. also performed metabolic assays and rescue experiments. Z.L. performed the immunoblot analyses. I.J. performed the metabolite analyses. D.K.G. and S.L. provided patient specimens and discussed the work. M.R.R. and L.J. conducted TCGA and TARGET database mining and performed the correlation analyses. S.K. and H.E.B. provided critical advice on experimental design and interpretation of the data, and edited the manuscript. C.K.Q. designed the experiments and directed the entire project. F.N. and C.K.Q. wrote the manuscript with input from all authors.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s42255-019-0039-6.
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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  - Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated
- Clearly defined error bars
  - State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

In flow cytometric analyses, data were collected on BD LSR II Flow Cytometer (BD Biosciences, NJ).

Data analysis

Statistical analyses were performed using GraphPad Prism v7.0; Flow cytometry analyses were performed using FlowJo v10 (Treestar, Ashland, OR).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The metabolics data supporting the findings of this study are available (Supplementary Table 1). We also provided the uncropped scans of the Western blots as Supplementary Figure 13. The data that support the findings of this study are available from the corresponding author upon request.
Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

Sample sizes were chosen according to similar studies reported in the literature. Three to eight (or more) mice per genotype or treatment group were used in independent experiments. We also consulted a statistician at the Biostatistics and Bioinformatics Shared Resources at Emory University and confirmed that these sample sizes would have sufficient power in two tailed Student’s t-test to determine statistical significance between two genotypes or treatments. All results were reported mean +/- SD from independent biological replicates. The number of the independent experiments was indicated in each figure legend.

Data exclusions

No data were excluded from the manuscript.

Replication

For each assessment/measurement, similar results were consistently obtained in multiple independent experiments with different biological replicates, and all experiments are reported in the manuscript.

Randomization

For all animal experiments, mice of the same age, sex, and genotype were mixed and then randomly grouped for subsequent analyses.

Blinding

Investigators were not blinded to the experiments. However, we followed standard laboratory procedures of randomization. Each experiment was associated with proper controls, and compared samples were collected and analyzed under the same conditions. The experimental observations would be consistent irrespective of blinding. Conclusions were made based on independent experiments, quantitative parameters and statistical significance of the data.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a Involved in the study
- Unique biological materials
- Antibodies
- Eukaryotic cell lines
- Palaeontology
- Animals and other organisms
- Human research participants

Methods

n/a Involved in the study
- ChIP-seq
- Flow cytometry
- MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials

Slc1a5 global and conditional knockout mice were generated in our own laboratory. They will be made available to all investigators after this report is published. There is no restriction on availability of other materials used in this study.

Antibodies

Antibodies used

Antibodies used in flow cytometry were anti-CD45.2 (BD Biosciences, 553772, clone 104,1/100 dilution), anti-CD45.1 (BD Biosciences, 12-0453-82, clone A20,1/100 dilution), anti-Mac-1 (BD Biosciences, 12-0112-83, clone M1/70,1/100 dilution), anti-Gr-1 (BD Biosciences, 12-5931-83, clone RB6-8C5,1/100 dilution), anti-CD3 (BD Biosciences, 553064, clone 145-2C11,1/100 dilution), anti-Ter119 (BD Biosciences, 12-0711-81, clone R17217,1/100 dilution), anti-CD71 (BD Biosciences, 11-0711-81, clone R17217,1/100 dilution), anti-c-Kit (BD Biosciences, 47-1171-82, clone 48-5931-82, clone RB6-8C5,1/100 dilution), anti-B220 (BD Biosciences, 12-0452-83, clone RA3-6B2,1/100 dilution), anti-CD3 (BD Biosciences, 553064, clone 145-2C11,1/100 dilution), anti-Ter119 (BD Biosciences, 12-5921-82, clone TER-119,1/100 dilution), anti-CD71 (BD Biosciences, 11-0711-81, clone R17217,1/100 dilution), anti-Sca-1 (BD Biosciences, 553612, clone D7,1/100 dilution), anti-c-Kit (BD Biosciences, 47-1171-82, clone 48-5931-82, clone RB6-8C5,1/100 dilution), anti-CD48 (BD Biosciences, 46-0481-80, clone HM48-1,1/100 dilution), anti-CD150 (Biolegend, 115918, clone TC15-12F12.2,1/100 dilution), anti-CD16/32 (BD Biosciences, 553144, clone 2.4G2,1/100 dilution), anti-CD127 (BD Biosciences, 15-1271-82, clone A7R34,1/100 dilution), anti-K67 (Biolegend, 151204, clone 11F6,1 μg/mL), anti-CD34 (BD Biosciences, 560230, clone RAM34,1/100 dilution), anti-human CD45 (BD Biosciences, 11-9459-42, clone 2D1,5 μL/test).
Antibodies used in Western blotting were ASCT2 (Cell Signaling Technology, 5345, clone V501, 1/1000 dilution), Phospho-AMPK (Cell Signaling Technology, 2535, clone 40H9, 1/1000 dilution), AMPK (Cell Signaling Technology, 5831, clone D5A2, 1/1000 dilution), Phospho-S6 Ribosomal Protein (Cell Signaling Technology, 4856, clone 2F9, 1/1000 dilution), S6 Ribosomal Protein (Cell Signaling Technology, 2217, clone 5G10, 1/1000 dilution), Phospho-p70 S6 Kinase (Cell Signaling Technology, 9208, 1/1000 dilution), p70 S6 Kinase (Cell Signaling Technology, 2708, clone 4D9F, 1/1000 dilution), Phospho-Akt (Ser473) (Cell Signaling Technology, 4060, clone D9E, 1/1000 dilution), Phospho-Akt (Thr308) (Cell Signaling Technology, 2965, clone C31E5E, 1/1000 dilution), Akt (pan) (Cell Signaling Technology, 4691, clone C67E7, 1/1000 dilution), PTEN (Cell Signaling Technology, 9559, 138G6, 1/1000 dilution), Phospho-mTOR (Cell Signaling Technology, 5536, clone D9C2, 1/1000 dilution), mTOR (Cell Signaling Technology, 2983, clone 7C10, 1/1000 dilution), phospho-ERK (Santa Cruz, sc-7383, clone E-4, 1/1000 dilution), ERK (Santa Cruz, sc-93, clone C-16, 1/1000 dilution), LC3A (Novus Biologicals, NB100-2331, 1/50 dilution) and beta-Actin (Santa Cruz, sc-47778, clone C4, 1/2000 dilution). Secondary antibodies were IRDye 680RD goat anti-mouse IgG (LI-COR, 92668070, 1/20000 dilution) and IRDye 800CW goat anti-rabbit IgG (LI-COR, 82708365, 1/20000 dilution).

Validation

The antibodies were chosen based on the experience in our own laboratory and/or the provider’s catalog information on the ones used in previously published studies in the literature. All antibodies were titered on control samples to determine the maximal dilution to achieve population separation without observable background.
Gating strategy

were first gated using forward scatter (FSC) versus side scatter (SSC). Viable cells were then gated on FSC versus viability dye staining. Boundaries between positive and negative staining were defined by using fluorescence minus one controls, following which specific populations were gated according to the defined cell surface phenotypes.

For hematopoietic stem cell (HSC) analyses, viable bone marrow cells were gated on SSC versus Lineage markers (CD3e, B220, Gr-1, CD11b, and Ter119). The Lin- cell population was then gated on Sca1 versus c-Kit. The Lin-Sca1+c-Kit+ (LSK) cell population was further gated on CD150 versus CD48. For progenitor assays, the Lin-c-Kit+ cell population and the Sca-1 low/c-Kit low cell population were gated as described above. These gated cell populations were further gated on CD34 versus CD16/32 or on side scatter versus CD127. For erythroid blast analyses, viable bone marrow cells were gated on CD71 versus Ter119. In competitive repopulation assays, peripheral blood and bone marrow cells were gated on CD45.1 versus CD45.2 or CD45.2 versus lineage markers. For cell cycle analyses, singlets were gated on FSC (height) versus FSC (area) and further gated on the HSC population as described above. This gated cell population was then gated on Ki67 versus hoechst 33342. For apoptosis analyses, the gated HSC population was further gated on Annexin V versus 7-AAD.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.