Cancer progression by reprogrammed BCAA metabolism in myeloid leukaemia

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Reprogrammed cellular metabolism is a common characteristic observed in various cancers1,2. However, whether metabolic changes directly regulate cancer development and progression remains poorly understood. Here we show that BCAT1, a cytosolic aminotransferase for branched-chain amino acids (BCAAs), is aberrantly activated and functionally required for chronic myeloid leukaemia (CML) in humans and in mouse models of CML. BCAT1 is upregulated during progression of CML and promotes BCAA production in leukaemia cells by aminating the branched-chain keto acids. Blocking BCAT1 gene expression or enzymatic activity induces cellular differentiation and impairs the propagation of blast crisis CML both in vitro and in vivo. Stable-isotope tracer experiments combined with nuclear magnetic resonance-based metabolic analysis demonstrate the intracellular production of BCAAs by BCAT1. Direct supplementation with BCAAs ameliorates the defects caused by BCAT1 knockdown, indicating that BCAT1 exerts its oncogenic function through BCAA production in blast crisis CML cells. Importantly, BCAT1 expression not only is activated in human blast crisis CML and de novo acute myeloid leukaemia, but also predicts disease outcome in patients. As an upstream regulator of BCAT1 expression, we identified Musashi2 (MSI2), an oncogenic RNA binding protein that is required for blast crisis CML. MSI2 is physically associated with the BCAT1 transcript and positively regulates its protein expression in leukaemia. Taken together, this work reveals that altered BCAA metabolism activated through the MSI2–BCAT1 axis drives cancer progression in myeloid leukaemia.

To understand the contribution of α-amino acid metabolism to the cancer progression of CML, we analysed blood amino acid levels in mouse models that recapitulate the chronic and blast crisis phases of human CML. Using amine-specific fluorescent labelling coupled with high-performance liquid chromatography (HPLC), 16 amino acids were quantified in the blood plasma from leukaemic mice (Extended Data Fig. 1a–d). Mice bearing blast crisis (BC)-CML showed moderate but significant elevations of plasma glutamate, alanine and the BCAAs (namely valine, leucine and isoleucine) compared with chronic phase (CP)-CML mice (Extended Data Fig. 1e). Intracellular levels of BCAAs and proline were higher in BC-CML, whereas intracellular glutamate and alanine were comparable in the two disease phases (Fig. 1a). These results suggest that increased BCAA uptake or metabolism may contribute to CML progression. We analysed the gene expression and found no significant upregulation of known BCAA transporters in BC-CML compared with CP-CML (data not shown). Leucine import into BC-CML cells was not greater than into CP-CML cells (Extended Data Fig. 1f), indicating that increased BCAA uptake does not explain the higher BCAA levels in BC-CML. To examine the possibility of altered intracellular BCAA metabolism, we next analysed the expression of genes encoding amino acid metabolic enzymes and found that the branched-chain amino acid aminotransferase 1 (Bcat1) was more highly expressed in BC-CML than in CP-CML at both the messenger RNA (mRNA) and protein levels (Fig. 1b–c and Extended Data Fig. 1g–h). In contrast, normal haematopoietic stem/progenitor cells (HSPCs) from healthy mice had very low levels of Bcat1 expression (Lin−Sca-1+ c-KIt) (LSK) population; Fig. 1b), and normal tissues did not show detectable Bcat1 expression except for the brain and testis (Extended Data Fig. 1i). Bcat1 encodes an evolutionarily conserved cytoplasmic aminotransferase for glutamate and BCAAs, constituting a regulatory component of cytoplasmic amino acid and keto acid metabolism (1,2). Bcat2, a parologue encoding the mitochondrial BCAA aminotransferase, and alanine and aspartate aminotransferases did not show differential expression between CP- and BC-CML (Extended Data Fig. 1g, k–l).

Although BCAT1 catalyses transamination in both directions, the breakdown of BCAAs is the predominant reaction in most cell types. For BCAT1 to generate BCAAs via the reverse reaction, the corresponding branched-chain keto acids (BCKAs), as well as glutamate, must be present as substrates. We found that all three BCKAs, keto-isovalerate (KIV), keto-isocaproate (KIC) and keto-methylvalerate were present in both the blood plasma and leukaemia cells (Extended Data Fig. 2a–d). In BC-CML cells, BCKAs were present at concentrations equivalent to 22–55% of the corresponding BCAAs, suggesting that intracellular BCKAs can serve as substrates for BCAA production (Extended Data Fig. 2e). Next, we examined whether BCAAs are produced through BCAT1 transamination reactions in leukaemia cells by stable-isotope tracer experiments with [13C]valine or [13C]KIV. Intracellular [13C]-labelled metabolites in K562 human BC-CML cells were analysed using one- and two-dimensional 1H–13C heteronuclear single-bond correlation (HSQC) analysis by high-field NMR spectroscopy (Fig. 1e–h and Extended Data Fig. 3). HSQC analysis detects only metabolites that have incorporated 13C isotope. To determine whether KIV is converted to valine, cells were cultured in media supplemented with uniformly labelled [(U)-13C]KIV and non-labelled valine at physiological concentrations (30 and 170 μM, respectively), and analysed for intracellular [13C]-labelled metabolites. After 15 min of labelling, the generation of [13C]valine was clearly observed, indicating the efficient intracellular production of valine from KIV (Fig. 1f, h). In contrast, [13C]KIV formation was barely detectable in the cells cultured with non-labelled KIV and [(U)-13C]valine (Fig. 1e, g). Our observation of intracellular [13C]valine signals indicates its transport into BC-CML cells. We also detected robust signals for [13C]KIV when present (Extended Data Fig. 3d, f). The formation of valine from KIV, but not the breakdown of valine to KIV, was also observed when we used equal concentrations of KIV and valine in the labelling media (170 μM each; Fig. 1g, h). We did not detect KIC formation from...
Activated BCAA production by BCAT1 in BC-CML.

**a.** Intracellular amino acid levels in CP-CML (n = 7, open bars) and BC-CML (n = 9, closed bars). Amounts per 2 × 10⁷ cells. B. cat1 expression in normal and leukaemic haematopoietic cells. Serial cDNA dilutions were used for reverse-transcription PCR (RT–PCR) analysis. Normal LSK cells, CP- and BC-CML cells, M1 myeloid cells and no reverse transcriptase (−RT) and water controls are shown. B2m, β-2-microglobulin.

c. BCAT1 protein expression in primary mouse leukaemia (n = 3 each).

d. Schematics of the reaction catalysed by BCAT1. KG, α-ketoglutarate;

[¹³C]leucine either (Extended Data Fig. 3g-i). These results indicate that little, if any, BCAs are catabolized to BCKAs in leukaemia cells.

To provide further evidence for the intracellular BCAA production through transamination, we performed alternative labelling experiments to track the fate of the amine group of glutamate. We cultured almost all the mice that were transplanted with control cells succumbed to the disease (line ‘1k’ in Fig. 2e). In contrast, normal HSPCs were only minimally affected by gene knockdown or Gbp treatment (Extended Data Fig. 5d). As an alternative approach to gene knockdown, we treated BC-CML cells with gabapentin (Gbp), a chemical inhibitor of BCAT1. Gbp is a structural analogue of leucine and specifically and competitively inhibits the transaminase activity of BCAT1 but not that of BCAT2 (ref. 7). BC-CML cells plated with Gbp formed smaller colonies and showed a dose-dependent impairment in clonogenic growth (Fig. 2b). In contrast, normal HSPCs were only minimally affected by gene knockdown or Gbp treatment (Extended Data Fig. 5e, f). These data suggest that BCAT1 inhibition may selectively impair the propagation of leukaemia without affecting normal haematopoiesis.

To examine whether Betat1 loss affects the propagation of BC-CML in vivo, Lin− cells expressing shBCat1 were transplanted into conditioned recipient mice. Whereas 75% of the recipients transplanted with control cells succumbed to the disease within 30 days, only 47% (shCat1-a) and 31% (shCat1-b) of the mice transplanted with Betat1-knockdown cells developed the disease, and more than half of these mice survived even when followed out to 60 days (Fig. 2c). Among the mice that developed disease with Betat1 knockdown, most had leukaemia that was characterized by differentiated granulocytes and lower levels of immature myeloblasts (Fig. 2d and Extended Data Fig. 5g). They also displayed a lower frequency of immature Lin− cells than control leukaemia (Extended Data Fig. 5h), indicating that the loss of Betat1 induced differentiation and impaired the leukaemia-initiating cell activity. Consistent with these phenotypes, serial transplantation of the leukaemia cells revealed that while all the control leukaemia cells propagated the disease, none of the mice transplanted with Betat1-knockdown leukaemia cells succumbed to the disease (line ‘1k’ in Fig. 2e). In addition, we established a doxycycline (Dox)-inducible Betat1 knockdown system (i-shBetat1) and examined the impact of Betat1 loss on the disease maintenance. Ten days after transplantation with BC-CML cells infected with i-shBetat1, leukaemic engraftment was assessed in each recipient, and Dox treatment was initiated (Extended Data Fig. 5i, j). While almost all the mice that were transplanted with control cells
and the non-Dox-treated mice developed leukaemia, more than half of the Dox-treated i-shBcat1 mice remained disease-free (Extended Data Fig. 5k), indicating that Bcat1 is required for the continuous propagation of BC-CML. At the cellular level, we did not observe enhanced apoptosis or a decrease in actively cycling cells by Bcat1 knockdown (Extended Data Fig. 5l, m). These results demonstrate that Bcat1 is critical for the sustained growth and maintenance of leukaemia-initiating cells in BC-CML.

We next examined whether the enforced expression of Bcat1 could drive blastic transformation in haematopoietic cells. Although we observed a significant increase in Bcat1 expression compared with the vector control, Bcat1 expression alone did not enhance the colony-forming ability of either LSK or Lin~ c-Kit~ haematopoietic cells isolated from normal bone marrow (Extended Data Fig. 6a, b). To determine whether BCR–ABL1 cooperates with Bcat1 overexpression to confer an aggressive growth phenotype, we transduced normal HSPCs with Bcat1 and BCR–ABL1. Compared with the vector control, the combinatorial expression promoted clonogenic growth in vitro (Extended Data Fig. 6c), and the transplantation of the cells led to significantly elevated leukaemia burdens (Extended Data Fig. 6d, e), splenomegaly and increased mortality in the recipient mice (Fig. 2f), with a concomitant increase in plasma BCAA levels (Extended Data Fig. 6f). Accordingly, leukaemia that developed in response to Bcat1 overexpression exhibited a highly immature myeloblastic morphology compared with the control (Fig. 2g and Extended Data Fig. 6g). These data indicate that activated Bcat1 mediates the blastic transformation of CP-CML cells.

Our results demonstrate that Bcat1 is essential for the development of BC-CML in mice, while normal bone marrow HSPCs show a very limited dependence on this metabolic enzyme. To investigate the contribution of BCAAT1 to human leukaemia, we looked at a panel of 13 peripheral blood samples from healthy and leukaemic individuals and found human BCAAT1 expression was higher in BC-CML than in either normal or CP-CML cells (Fig. 3a). To determine whether this expression pattern reflects a general trend in human CML, we analysed BCAAT1 levels in a Gene Expression Omnibus (GEO) dataset of 113 cases of CML. This focused analysis revealed a significant elevation in BCAAT1 expression as the disease progresses from the chronic to the accelerated phase and then to the blast crisis phase (Fig. 3b). On average, BCAAT1 expression was 15-fold higher in BC-CML than in CP-CML. We did not find significant changes in BCAAT2 expression, which is consistent with the results from the mouse models (Fig. 3c and Extended Data Fig. 1g). These data indicate that activation of BCAAT1 is a shared characteristic in the progression of human CML.

Lentiviral BCAAT1 knockdown or Gbp treatment markedly inhibited the colony-forming ability of K562 human BC-CML (Extended Data Fig. 7a–d) and patient-derived primary leukaemia cells (Fig. 3d, e and Extended Data Fig. 7e, f). Interestingly, we observed BCAAT1 activation in primary human acute myeloid leukaemia as well (AML; Fig. 3f), and Gbp effectively inhibited the clonal growth of human AML cell lines and primary de novo AML cells (Fig. 3g and Extended Data Fig. 7g–i). Moreover, BCAAT1 expression levels predict disease outcome in patient cohorts. Cases from The Cancer Genome Atlas (TCGA) AML dataset were divided into quartiles on the basis of BCAAT1 expression levels (Extended Data Fig. 7j), and we found that the median survival time was 46% shorter in the BCAAT1-high group (427 versus 792 days; Fig. 3h). These results demonstrate an essential role for BCAAT1 in the pathogenesis of a wide array of human myeloid malignancies.

To understand how the BCAAT1-driven change in metabolism promotes leukaemia growth, we analysed intracellular amino acid concentrations upon BCAAT1 inhibition and found that all three BCAs were significantly reduced by shBCAT1 or Gbp treatment compared with the controls (Extended Data Fig. 8a, b). Interestingly, the addition of BCAs, but not alanine–glutamine (GlutaMax), functionally suppressed the reduction of colony-forming ability caused by BCAAT1 knockdown (Fig. 3i), suggesting that BCAAT1 enhances clonogenic growth through BCAA production via BCKA reamination. BCAs, particularly leucine, activate the mTORC1 pathway via cytosolic leucine sensor proteins, which integrate multiple signals from nutrient sensing and growth factor stimuli to promote cell growth. Thus, we examined whether reduced BCAA production by BCAAT1 inhibition results in the attenuation of the mTORC1 signal. Indeed, BCAAT1 knockdown by either shRNA or Gbp treatment significantly reduced the phosphorylation of S6 kinase (pS6K), a downstream target of mTORC1 kinase (Fig. 3j), suggesting BCAAT1 activation of the mTORC1 pathway. We observed no apparent changes in the levels of phosphorylated AKT upon BCAAT1 inhibition, suggesting a predominant contribution of BCAA nutrient signals to the activation of mTORC1 (Extended Data Fig. 8c). Consistently, the mTORC1 inhibitor rapamycin reversed the BCAA-induced suppression of colony formation (Fig. 3i) and the BCAA-induced increase in pS6K (Fig. 3k).

To further investigate the BCAAT1-mediated regulation of CML progression, we performed gene correlation analyses using tumour gene expression datasets available in the GEO and TCGA databases. We found that BCAAT1 and MSI2 are often co-expressed in several types of cancer, including leukaemias, colorectal and breast cancers (Extended Data Fig. 9a, b). MSI2 is a member of the evolutionarily conserved Musashi RNA binding protein family, which regulates cell...
BCAT1 translation and mTORC1 activation. Importantly, BCAT1 overexpression (Fig. 4c) and BCAA supplementation (Fig. 4d) effectively suppressed the attenuation of the colony-forming ability caused by MSI2 knockdown, with a concomitant increase in pS6K levels in a

a. Kaplan–Meier analysis of overall survival in the AML patient cohorts with low (bottom quartile) or high (top quartile) BCAT1 expression.

b. Kaplan–Meier analysis of overall survival in the AML patient cohorts with low (bottom quartile) or high (top quartile) BCAT1 expression.

c. Kaplan–Meier analysis of overall survival in the AML patient cohorts with low (bottom quartile) or high (top quartile) BCAT1 expression.

d. Kaplan–Meier analysis of overall survival in the AML patient cohorts with low (bottom quartile) or high (top quartile) BCAT1 expression.

Figure 3 | BCAT1 activation and requirement in human myeloid leukaemia. a, BCAT1 expression in healthy individuals (n = 5) and in patients with CP- and BC-CML (n = 4 each) at The Institute of Medical Science Hospital, University of Tokyo. b, c, Microarray data analysis of (b) BCAT1 and (c) BCAT2 expression in 57 chronic (grey), 15 accelerated (pink) and 41 blast crisis (blue) phase patients. Bars, normalized expression in each specimen. d, e, Colony-forming ability of primary human BC-CML cells treated with (d) shBCAT1 or (e) Gbp. n = 3 each. Two independent patient specimens were tested. f, BCAT1 expression in healthy subjects and patients with de novo AML at The Institute of Medical Science Hospital, University of Tokyo (n = 5).

g. Colony-forming ability of Gbp-treated primary human AML cells.
rapamycin-sensitive manner (Fig. 4e). The levels of AKT phosphorylation were unaffected by shMSI2 (Extended Data Fig. 8c). Collectively, our work presented here demonstrates an essential role for the MSI2–BCAT1 axis in myeloid leukaemia and provides a proof-of-principle for inhibiting the BCAA metabolic pathway to regulate CML progression (Fig. 4f).

The upregulation and functional requirements of BCAT1 have been reported in glioblastoma and in colorectal and breast tumours.22,23 Interestingly, Musashi proteins also regulate the same spectrum of cancers including myeloid leukaemia18–21,24,25, suggesting a highly conserved role for the MSI–BCAT1 pathway in multiple cancer types. Despite the conservation of this pathway, the metabolic role of BCAT1 seems distinct and dependent on the tissue of origin; in the brain, BCAT1 catalyses BCAA breakdown and glutamate production to enhance tumour growth in glioblastoma, whereas it promotes BCAA production in leukaemia. Two different types of tumour, specifically pancreatic ductal adenocarcinoma and non-small-cell lung carcinoma, were recently shown to exhibit different usages of BCAs26. Despite the same initiating events of KRAS activation and TP53 deletion, non-small-cell lung carcinoma cells actively utilize BCAs by enhancing their uptake and oxidative breakdown to BCKAs, whereas pancreatic ductal adenocarcinoma cells display decreased uptake and thus little dependency on BCAs. Consistently, BCAT1 and BCAT2 are required for tumour formation in non-small-cell lung carcinoma but not in pancreatic ductal adenocarcinoma. Although BCAT1 is functionally required for tumour growth in a broad range of malignancies, these reports and our studies highlight the context-dependent role of the BCAT1 metabolic pathway in cancer.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions A.H. designed the studies, performed all experiments, analysed the data and wrote the manuscript. M.T. designed and performed experiments related to quantitative analysis of amino and keto acids. T.K., M.K. and A.T. provided and performed experiments with human primary samples. T.N. performed histological and cytoligical analysis. J.G., F.T. and A.S.E. designed and conducted NMR-based metabolic analysis. D.M. and N.K. performed bioinformatics analysis of gene expression datasets. T.I. conceived and supervised the project and wrote the manuscript.

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**METHODS**

**Mice.** C57BL/6 mice were from the Jackson Laboratory. Mice were bred and maintained in the facility of the University Research Animal Resources at University of Georgia. All mice were 8–16 weeks old, age- and sex-matched and randomly chosen for experimental use. No statistical methods were used for sample size estimates. All animal experiments were performed according to protocols approved by the University of Georgia Institutional Animal Care and Use Committee. The investigators were not blinded to allocation during experiments and outcome assessment.

**Cell isolation, analysis and sorting.** Cells were suspended for cell sorting in Hank’s balanced salt solution (HBSS) containing 5% (v/v) fetal bovine serum (FBS) and 2 mM EDTA as previously described\(^{29}\). The following antibodies were used to define lineage positive cells: 145-2C1 (CD13), GK1.5 (CD4), 53-6.7 (CD8), RB6-8C5 (Ly-6G/Gr1), M1/70 (CD11b/Mac-1), TER119 (Ly-76/TER119), 662B (CD45R/B220) and eBio1D3 (CD19). Red blood cells were lysed with RBC Lysis Buffer (BioEiscience) before staining for lineage markers. For the LSK bone marrow cell sorting, the antibodies 2B8 (c-Kit/CD117) and D7 (Sca-1/Ly-6A/E) were also used. To determine donor-derived chimaerism in the transplantation-based assays, peripheral blood from the recipients was obtained by the subman dibular bleeding method and prepared for analysis as previously described\(^{29}\). All antibodies were purchased from eBioscience. Apoptosis assays were performed by staining cells with Annexin V and 7-AAD (BioLegend). Cell cycle status was analysed for donor chimaerism by staining cells with Annexin V and 7-AAD (BioLegend). Cell cycle status was analysed by staining cells with 2.5 μg ml\(^{-1}\) PI containing 0.1% BSA and 2 μg ml\(^{-1}\) RNase after fixation with 70% ethanol. Flow cytometric analysis and cell sorting were performed on the MoFlo XDP, Cyan ADP (Beckton Coulter) or S3 (Bio-Rad), and the data were analysed with FlowJo software (Tree Star).

**Viral constructs and production.** Retroviral BCR–ABL1 and NUP98–HOXA9 vectors and lentiviral FG2-IUBc-GFP vector were obtained from Addgene. Mouse Bcat1 DNA (IMAGE clone ID 30061662) was cloned into MSCV–IRS–GFP and Human BCA1 CDNA (NITE clone ID AK056255) was cloned into FG2-Iub-hcCD2. The short hairpin RNA constructs against Bcat1 (shBcat1) were designed and cloned in MSCV-ITRMiR30-PIG (LMP) vector from Open Biosystems or TtRMPVIR from Addgene according to their instructions. The target sequences are 5′-CCCGAGGTGTGACCCAGA-3′ for shBcat1-a, 5′-TCCGGCCGCTTGTTGAGAAA-3′ for shBcat1-b and 5′-CTGGTCCAGATCTCTTCAGTAG-3′ for lucerase as a negative control (shCtrl). Lentiviral short hairpin RNA (shRNA) constructs were cloned in FG2IUB1 essentially as described previously\(^{29}\). The target sequences are 5′-GCAAGGATGTGACCCAGA-3′ for shBcat1-c, 5′-TGGCCCATGTTAGGACCGT-3′ for shBcat1-d and 5′-TGCCTGCTGTGCTGCCA-3′ for a negative control. Virus was produced in HEK 293FT cells transfected using polyethylenimine with viral constructs along with VSVG and gag-pol. For lentivirus production Rev was also co-transfected. Viral supernatants were collected for two days followed by ultracentrifugal concentration at 50,000g for 2 h.

**Cell culture and colony formation assays.** The human BC-CML cell line K562, the human acute leukaemia cell lines MV4-11 and U937 were maintained in Roswell Park Memorial Institute 1640 medium (RPMI-1640) with 10% FBS, 100 IU ml\(^{-1}\) penicillin and 100 μg ml\(^{-1}\) streptomycin, 55 μg ml\(^{-1}\) 2-mercaptoethanol, 10% FBS, 1% L-glutamine and 1% non-essential amino acids in a humidified 5% CO\(_2\) incubator at 37°C. The BCR–ABL–IRES–YFP and MSCV–BCR–ABL–IRES–GFP, and doubly infected cells were purified by fluorescence-activated cell sorting and transplanted into recipients that were sublethally irradiated. For Bact1 knockdown by retroviral shRNA transduction, the Lin− population from BC-CML cells was sorted and infected with either control shCtrl (against luciferase) or shBcat1-a/b (against Bcat1) retrovirus for 48 h. Infected cells were sorted on the basis of GFP expression, and 1,000 or 2,000 cells were transplanted in sublethally irradiated C57BL/6J recipients. For conditional Bcat1 knockdown by a Dox inducible shRNA system, animals were analysed for donor chimaerism at day 10 after transplantation, and then Dox treatment was initiated by feeding Dox-containing rodent chow (0.2 mg g\(^{-1}\) diet; S8888, BioServ). After transplantation, recipient mice were maintained on antibiotic water (sulfamethoxazole/trimethoprim) and evaluated daily for signs of morbidity, weight loss, failure to groom and splenomegaly. Premorbid animals were euthanized, and relevant tissues were harvested and analysed by flow cytometry and histopathology. For secondary BC-CML transplants, mice recovered from terminally ill primary recipients were sorted for Lin− donor cells and transplanted into secondary recipients. Where indicated, sorted live BC-CML cells from the spleen were cryopreserved and stained with Wright’s stain solution (Harleco) for cyto-pathologic evaluation by a board-certified veterinary pathologist (TN.).

**Primary human leukaemia samples.** Blood samples from patients were obtained at the Institute of Medical Science Hospital, University of Tokyo, with written informed consent according to the procedures approved by the Institutional Review Board. Mononuclear cells from the individuals were viability frozen and stored in liquid nitrogen. For in vitro colony formation with Bcat1 knockdown, primary HCD34+ cells sorted from patients’ bone marrow samples were cultured in IMDM supplemented with 10% FBS, 100 IU ml\(^{-1}\) penicillin and 100 μg ml\(^{-1}\) streptomycin, 55 μg ml\(^{-1}\) 2-mercaptoethanol, stem cell factor, IL-3, IL-6, FL/T3 and thrombopoietin. After 24 h of culture, the cells were transduced with lentiviral shRNA (cloned in FG2-IUBc-GFP), and the GFP-positive infected cells were sorted at 48 h, and 5,000–50,000 cells were plated in complete methylcellulose medium (Methocult H4435, StemCell Technologies). For the colony-formation assays with Gbp, sorted hCD34+ cells from the primary patient specimens were cultured in complete methylcellulose medium with the indicated concentrations of Gbp. Colonies were scored on days 9–14.

**Bioinformatic analysis of human gene expression.** For the focused gene expression analysis of Bcat1- and MSi2 across multiple cancer types, the GEO datasets GSE14671 (CML), GSE10327 (medulloblastoma), GSE0916 (cerebro), GSE14548 (breast) and TCGA datasets LAML (AML) and LUAD (lun adenocarcinoma) were collected and analysed in a similar fashion.

**Real-time and standard RT–PCR analysis.** Total cellular RNAs were isolated using an RNAsqueous-Micro kit (Ambion), and CDNAs were prepared from equal amounts of RNAs using Superscript III reverse transcriptase (Life Techno logies). Real-time PCR primers were designed and ordered at SBA (Taq PCR Master Mix (Life Technologies), cDNA and 0.5 μM of each primer. PCR conditions were as follows: 1 min at 94°C followed by 35 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s. PCR primer sequences were as follows: B2m–F1, 5′-ACCAGGCTTGTATGCTATCCAGAAA-3′; B2m–R1, 5′-CTGATGGTGATCCTGAGCAAC-3′; Bcat1–F1, 5′-TTGTTGCTTAGGCAAGTGGAC-3′; Bcat1–R1, 5′-GTAATGTCGGCTGTCCAGT-3′. Real-time PCR were performed using EvaGreen qPCR Master Mix (Bio-Rad) on an iQ5 (Bio-Rad), or using TaqMan Gene Expression Assays on an Applied Biosystems 7500 Real-Time PCR System (Life Technologies). Results were normalized to the level of 18S–microglobulin. PCR primer sets were designed as follows: hMYC-R, 5′-ATTGTCCAGTCGCTC-3′ and hMYC-F, 5′-ATTGTCCAGTCGCTC-3′. hMYC-R was designed and ordered at SBA (Taq PCR Master Mix (Life Technologies), cDNA and 0.5 μM of each primer. PCR conditions were as follows: 1 min at 94°C, followed by 35 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s. PCR primer sequences were as follows: B2m–F1, 5′-ACCAGGCTTGTATGCTATCCAGAAA-3′; B2m–R1, 5′-CTGATGGTGATCCTGAGCAAC-3′; Bcat1–F1, 5′-TTGTTGCTTAGGCAAGTGGAC-3′; Bcat1–R1, 5′-GTAATGTCGGCTGTCCAGT-3′. Real-time PCR were performed using EvaGreen qPCR Master Mix (Bio-Rad) on an iQ5 (Bio-Rad), or using TaqMan Gene Expression Assays on an Applied Biosystems 7500 Real-Time PCR System (Life Technologies). Results were normalized to the level of 18S–microglobulin. PCR primer sets were designed as follows: hMYC-R, 5′-ATTGTCCAGTCGCTC-3′ and hMYC-F, 5′-ATTGTCCAGTCGCTC-3′.
analysed with TaqMan Gene Expression Assays: Bcat1 (Mm00500289_m1), Bcat2 (Mm00802192_m1), Gpt1 (Mm00805379_g1), Gpt2 (Mm00558028_m1), G0t1 (Mm0049698_m1), G0t2 (Mm0049703_m1).

**Amino acid and keto acid quantification.** Leukaemia cells or peripheral blood samples drawn from mice bearing myeloid leukaemia were used for amino acid and keto acid analysis by HPLC-fluorescence detection. Typically, 200,000 live leukaemia cells per sample were sorted and washed twice with ice-cold PBS to remove media components before extraction. The blood plasma was prepared by centrifugation of the peripheral blood samples at 2,000g for 4°C for 10 min. Plasma fractions were then treated with 45% methanol/45% acetonitrile containing 6-aminoisopropyl acid (internal standard for amino acid analysis) and α-ketovalerate (internal standard for keto acid analysis) on ice for 10 min. Cell samples were treated with 80% methanol instead of the 45% methanol/acetonitrile mixture. After removing the insoluble particles by centrifugation, the supernatants were collected and dried using a SpeedVac at 30°C. The 1H data were collected with a 20-s relaxation and TOCSY) were also collected in some cases to confirm assignments. The data were ing 0.1 mM DSS. All signals were identified either with authentic samples or by

The cells were subsequently washed twice with cold HBSS and lysed using 100 mM containing 10 mM citrate buffer. The concentrations are based on the standard RPMI-1640 media formulation. At

The mobile phases included (A) 10 mM citrate buffer containing 75 mM sodium perchlorate (pH 6.2) and (B) water/acetonitrile (50/50, v/v). The gradient conditions were as follows: t = 0 min, 100 B; t = 20 min, 50% B; and t = 30 min, 100% B. For NBD–Asn, Ser, Thr and Phe analysis, 10 mM citrate buffer containing 75 mM sodium perchlorate (pH 4.4) was used as the mobile phase A. NBD–amino acids were detected with excitation and emission wavelengths of 470 and 530 nm, respectively. For keto acid quantification, drie samples were treated with o-phenylenediamine (OPD) to derivatize α-keto acids, followed by liquid–liquid extraction with ethyl acetate. HPLC separation of OPD–keto acids was performed on an Inertsil ODS-4 column (3.0 mm × 250 mm, 5 μm, GL Sciences, Tokyo, Japan) at a flow rate of 0.6 ml min⁻¹.

The mobile phase was water/methanol (55/45, v/v, Fluorescence detection was performed at the emission wavelength of 415 nm with excitation at 360 nm. **Measurement of leucine uptake in primary mouse leukaemia cells.** Primary mouse leukaemia cells from the spleens of the mice bearing myeloid leukaemia were used for measurement of leucine uptake and processed for 6h (GraphPad Software). Data are shown as the mean ± s.e.m. Two-tailed Student’s t-tests or Mann–Whitney U-tests were used to determine statistical significance. For Kaplan–Meier survival analysis, log-rank tests were used for statistical significance (*P < 0.05, **P < 0.01, ***P < 0.001).

**Data availability.** Source gel images and Source Data for animal models are included in the Supplementary Information. All NMR spectral data from metabolic analyses have been deposited in Metabolomics Workbench under the project identifier PR0000423. All other relevant data are available from the corresponding author upon request.

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Extended Data Figure 1 | Change in the amino acid metabolism in leukaemic mice. a–d, Representative chromatograms of (a, c) CP-CML and (b, d) BC-CML plasma samples derivatized with the amine-specific fluorescent labelling agent NBD-F and analysed in mobile phases at (a, b) pH 6.2 or (c, d) pH 4.4. Each NBD–amino acid peak was assigned as indicated. IS, internal standard (NBD–6-aminocaproic acid). e, Plasma amino acid levels in mice with CP- and BC-CML. Blood plasma samples were prepared from mice with CP- and BC-CML, methanol-extracted and dried under a vacuum. The dried extracts were analysed for quantification. n = 5 and 3, respectively. Error bars, s.e.m. **P < 0.01, ***P < 0.001. f, Leucine transport in primary CP- and BC-CML cells. BCR–ABL1–YFP<sup>+</sup> PI<sup>-</sup> live leukaemia cells (5 × 10<sup>6</sup>) were sorted from premorbid animals and placed in a pre-warmed uptake media containing 10 μM [(U)-<sup>14</sup>C]-leucine. After incubation at 37 °C for the indicated times, the uptake was determined. Error bars, s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001. g, RT–qPCR analysis of Bcat1 and Bcat2 expression in CP- and BC-CML cells (n = 4 each). The expression levels are normalized and displayed relative to the control β2-microglobulin gene expression. Error bars, s.e.m.; ***P < 0.001, NS, not statistically significant (P > 0.05). h, BCA1 protein expression in mouse primary CP- and BC-CML cells. This graph shows BCA1 protein expression levels normalized relative to the HSP90 loading control (CP-CML, n = 7; BC-CML, n = 9). Error bars, s.e.m. *P < 0.05. i, Tissue-specific expression of mouse Bcat1. The expression was detectable in the myeloid cell line M1, primary mouse BC-CML cells, olfactory bulb (Olf bulb), whole brain and testis. B2m, β2-microglobulin. j, Schematics of the structures of human and mouse BCA1 proteins. The shaded boxes represent aminotransferase domains. K, a Lys residue for the binding of the pyridoxal phosphate cofactor. CVVC, a conserved redox-sensitive CXXC motif. Regions targeted with shRNAs in this study are shown as thick bars (shBcat1-a and -b, and shBCAT1-c and -d). k, l, Alanine and aspartate transaminase gene expression in CP- and BC-CML. RT–qPCR analysis of (k) Gpt1 and Gpt2, and (l) Got1 and Got2 expression in CP- and BC-CML samples (n = 4 each). The expression levels are normalized and displayed relative to the expression of the B2m control. Error bars, s.e.m.; NS, not statistically significant (P > 0.05).
Extended Data Figure 2 | Keto acid metabolism in leukaemic mice.

a, b, Representative chromatograms of (a) CP-CML and (b) BC-CML plasma samples derivatized with the keto acid-reactive OPD. Each OPD–keto acid peak was assigned as indicated. KG, α-ketoglutarate; PYR, pyruvate; KIV, keto-isovalerate; KIC, keto-isocaproate; KMV, keto-methylvalerate; IS, internal standard for keto acid analysis (OPD–α-ketovalerate).

c, d, Plasma and intracellular branched-chain keto acid levels in CP- and BC-CML. c, d, Blood plasma fractions from leukaemic mice (c) or $5 \times 10^6$ live leukaemia cells purified by fluorescence-activated cell sorting (d) were methanol-extracted and dried under a vacuum. The dried extracts were labelled with OPD, extracted with ethyl acetate and analysed using an HPLC system equipped with a fluorescence detector. Open and closed bars indicate CP-CML (plasma, $n = 9$; intracellular, $n = 5$) and BC-CML (plasma, $n = 10$; intracellular, $n = 6$) specimens, respectively. *$P < 0.05$. Error bars, s.e.m.

e, Molar amount of intracellular BCAAs and BCKAs in primary mouse BC-CML cells. The amount of each organic acid per one million cells was estimated using calibration curves obtained with reference standards for each compound. %KA/AA shows the amount of a BCKA relative to the corresponding BCAA species.
Extended Data Figure 3 | Intracellular BCAA production from BCKA in human K562 BC-CML cells. a–f, Regions of HSQC spectra of $^{13}$C-labelled metabolites. K562 cells were cultured in media supplemented with (a, c) 170μM $[^{13}$C]valine and 30μM non-labelled KIV, or (b, d) 170μM non-labelled valine and 30μM $[^{13}$C]KIV. After labelling for 15 min, the cells were collected, washed with PBS and methanol-extracted for HSQC analysis by high-field NMR spectroscopy. Each panel shows the regions of one- and two-dimensional HSQC spectra for the (a, b) intracellular fraction, (c, d) culture supernatant and (e, f) labelling media alone, respectively. Panels a and b are the same as shown in Fig. 1e and f, respectively. g–i, Absence of detectable intracellular KIC generation by leucine breakdown. K562 cells were cultured in the labelling medium supplemented with 170μM $[^{13}$C]leucine and 30μM non-labelled KIC for 15 min, and the intracellular $^{13}$C-labelled metabolites were analysed by HSQC analysis. Each panel shows region of the two-dimensional spectrum showing $^1$H–$^{13}$C HSQC signals for β, γ and δ carbons of Leu and KIC. g, Intracellular fraction. (h) KIC reference standard (HSQC signals derived from natural abundance $[^{13}$C]KIC), (i) overlay of the spectra in g (black) and h (red). Note the absence of KIC signals in g.
Extended Data Figure 4 | Intracellular BCAA production via transamination. a–c, Regions of 600 MHz two-dimensional heteronuclear multiple-bond correlation spectra showing cross-peaks between the amine nitrogen and the β carbon protons. Only those amino acids that incorporated a significant amount of [15N]amine show cross-peak signals. d–f, Regions of 600 MHz one-dimensional [1H spectra. Each proton peak is assigned as indicated. DSS, 2,2-dimethyl-2-silapentane-5-sulfonate.

|          | % [15N]-AA/total AA |
|----------|---------------------|
| Val      | 24.3 ± 2.7          |
| Leu      | 38.9 ± 4.6          |
| Ile      | 33.7 ± 5.7          |
| total BCAAs | 34.6 ± 4.4        |

a, d, Mixture of reference standards of the indicated amino acids. b, e, K562 cell sample cultured in the medium containing (amine-[15N])-glutamine. c, f, K562 cell sample cultured in the non-labelled standard medium. g, Percentage of newly synthesized [15N]-labelled BCAAs within total intracellular pool at 72 h after post-labelling for each amino acid indicated. ‘Total BCAAs’ shows the percentage including all three BCAA species.
Extended Data Figure 5 | See next page for caption.
Extended Data Figure 5 | Roles of Bcat1 in differentiation, proliferation and leukaemia development in vivo. a, RT–qPCR analysis of Bcat1 expression. Lin− cells from NUP98–HOXA9/BCR–ABL-induced BC-CML were infected with shCtrl or Bcat1 shRNA (shBcat1-a and shBcat1-b) for three days and resorted for analysis of Bcat1 expression. The expression levels are normalized to the level of B2m expression and displayed relative to the control, which was arbitrarily set at 1. Error bars, s.e.m. of triplicate PCRs. **P < 0.01. b, RT–qPCR analysis of Bcat1 expression in leukaemia cells isolated from diseased mice transplanted with shCtrl- or shBcat1-expressing BC-CML cells. The expression levels are normalized to the level of B2m and are displayed relative to the control arbitrarily set at 1. Error bars, s.e.m. of triplicate PCRs. NS, not statistically significant (**P < 0.001). c, Bcat2 expression in shBcat1-expressing cells. Lin− cells from NUP98–HOXA9/BCR–ABL-induced BC-CML were infected with shCtrl or Bcat1 shRNA (shBcat1-a and shBcat1-b) for three days and resorted for analysis of Bcat2 expression. The expression levels are normalized to the level of B2m and are displayed relative to the control arbitrarily set at 1. Error bars, s.e.m. of triplicate PCRs. NS, not statistically significant (P > 0.05). d, Functional rescue of the shBcat1-induced reduction in colony-forming ability with the expression of shRNA-resistant mutant Bcat1 cDNA. Primary Lin− BC-CML cells transduced with the vector or shRNA-resistant Bcat1 gene together with the indicated shRNA constructs. **P < 0.01 compared with the vector and shBcat1-b. e, f, Colony-forming ability of primary HSPCs. Normal HSPCs purified from bone marrow on the basis of their LSK phenotype were transduced with the Bcat1 shRNAs (shBcat1-a and shBcat1-b) and plated for colony formation. NS, not statistically significant (P > 0.05). f, Normal HSPCs were plated for colony formation with the indicated concentrations of gabapentin or PBS (–). NS, not statistically significant (P > 0.05). **P < 0.01 compared with the PBS control. Photomicrographs showing representative colonies formed under each condition. Scale bars, 500 μm. Three hundred LSK cells were plated per well in triplicate for the evaluation of colony-forming activity. Error bars, s.e.m. g, Haematoxylin and eosin staining of sections of the liver, lung and spleen at the time of onset of clinical signs (top six rows) and of tissue sections from a disease-free survivor (bottom two rows). White arrows indicate immature myeloid cells. Portal triad (PT), haemorrhagic necrosis (N), central veins (CV), arteriolar profiles (A), bile ducts (B), veins (V), white pulp (WP) and red pulp (RP) are indicated. Scale bars, 100 μm for images at ×10 and 20 μm for images at ×40 magnification. h, Representative flow cytometry plots showing lineage marker expression in leukaemia cells from mice transplanted with the shRNA-infected BC-CML cells. Leukaemia cells were analysed for their frequency of the Lin− population. i–k, Effect of conditional Bcat1 knockdown on BC-CML maintenance in vivo. i, Lin− BC-CML cells were infected with doxycycline-inducible shRNAs against shBcat1-b or a control (shCtrl) and transplanted into recipients (1,500 cells per recipient). After 10 days of the transplantation with leukaemia cells expressing the indicated constructs, (j) donor-derived chimaerisms were analysed. Mice were then fed with chow containing doxycycline to induce shRNA expression, and (k) survival was monitored. The data shown are from two independent experiments. n = 4 for shCtrl with no Dox (DOX−); n = 7 for shBcat1-b, DOX−; and n = 9 each for shCtrl with Dox (DOX+) and shBcat1-b, DOX+. **P < 0.01 (shCtrl versus shBcat1-b, DOX+). NS, not statistically significant (P > 0.05). l, Cell cycle distribution of the shRNA-infected leukaemia cells. Live leukaemia cells were isolated from mice transplanted with shRNA-infected BC-CML cells, fixed and stained with propidium iodide for analysis of cell cycle distribution via flow cytometry. m, Apoptotic cells from leukaemic mice transplanted with shRNA-infected BC-CML cells were analysed via flow cytometry using Annexin V and 7-aminoactinomycin D (7-AAD) staining.
**Extended Data Figure 6** | **BCAT1** cooperates with **BCR–ABL1** in blastic transformation in vivo.  

**a**, RT–qPCR analysis of **Bcat1** expression in normal LSK or Lin<sup>−</sup>c-Kit<sup>+</sup> HSPCs transduced with either the vector or **Bcat1** retroviruses. The expression levels are normalized and displayed relative to the control **B2m** expression. **b**, Normal LSK or Lin<sup>−</sup>c-Kit<sup>+</sup> HSPCs were purified from healthy bone marrow and transduced with the indicated retroviruses, and the infected cells were plated in triplicate to assess colony formation after 10 days. Error bars, s.e.m. NS, not statistically significant (P > 0.05). **c**, Colony-forming ability of normal HSPCs expressing **BCR–ABL1** and **Bcat1**. LSK cells were purified from healthy bone marrow and transduced with either the control vector or **Bcat1** together with **BCR–ABL1** (B/A) retroviruses, and double-positive cells were plated in triplicate to assess colony formation after 10 days (plated at a density of 150 cells per well). Photomicrographs show representative colonies formed in each group. Scale bar, 500 μm. Error bars, s.e.m. **d**, Chimaerism of donor-derived cells after transplantation with LSK cells expressing the indicated constructs. n = 15 for each group. **e**, Haematoxylin and eosin staining of liver, lung and spleen sections from mice transplanted with LSK cells expressing **BCR–ABL1** and vector or **Bcat1**. White arrows indicate immature myeloid cells. Scale bars, 100 μm for ×10 images and 20 μm for ×40 images. **f**, Plasma α-amino acid levels in mice transplanted with LSK cells infected with **BCR–ABL1** and the vector or **Bcat1**. Blood plasma fractions were prepared from peripheral blood samples, methanol-extracted and dried under a vacuum. The dried extracts were labelled with NBD-F and analysed using an HPLC equipped with a fluorescence detector. Open and closed bars indicate vector control (n = 17) and **Bcat1** (n = 18) specimens, respectively. **g**, Representative flow cytometry plots showing lineage marker expression in leukaemia cells from mice transplanted with LSK cells infected with either the control vector or **Bcat1** together with **BCR–ABL1**. Leukaemia cells were analysed for their frequency of the Lin<sup>−</sup> population.
Extended Data Figure 7 | BCAT1 is required for human myeloid leukaemia. a, RT–qPCR analysis of BCAT1 expression in the human K562 BC-CML cell line transduced with lentiviral shCtrl or BCAT1 shRNA (shBCAT1-c and shBCAT1-d). The expression levels are normalized and displayed relative to the expression of the B2M control. **P < 0.01. b, Western blot analysis of BCAT1 protein levels in K562 cells infected with the indicated lentiviral shRNA constructs. Human β-tubulin protein (β-Tub) was used as the loading control. β-Tubulin image is the same as shown in Fig. 3j. c, d, Colony-forming ability of (c) K562 cells transduced with control (shCtrl) or BCAT1 shRNAs (shBCAT1-c and shBCAT1-d) and (d) K562 cells cultured with the indicated concentrations of Gbp. One hundred cells were plated per well in triplicate. Photomicrographs show representative colonies formed. Scale bar, 200μm. Error bars, s.e.m. **P < 0.01, ***P < 0.001. e, RT–qPCR analysis of BCAT1 expression in the samples from the patient with BC-CML used in the data presented in Fig. 3d that were transduced with control (shCtrl) or BCAT1 shRNA (shBCAT1-d). ***P < 0.001. f, Colony-forming ability of primary human CD34+ BC-CML cells from another specimen from a patient treated with Gbp. Error bars, s.e.m. **P < 0.01. g–i, Colony-forming ability of (g) MV4-11, (h) U937 and (i) HL60 human AML cells treated with the indicated concentrations of Gbp. MV4-11, HL60 cells (300 per well) or U937 (100 per well) were plated in triplicate. Photomicrographs show representative colonies formed. Scale bars, 200μm. Error bars, s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001. j, BCAT1 expression in human patients with de novo AML. Data for BCAT1 expression levels from the TCGA AML dataset were divided into quartiles and compared. On average, the top quartile cohort showed 1.6-fold higher expression level than the bottom quartile. **P < 0.01.
Extended Data Figure 8 | Impact of BCAT1 knockdown in K562 cells. 

**a, b.** Effect of (a) BCAT1 knockdown or (b) Gbp treatment on the intracellular concentrations of glutamate and BCAAs in K562 cells. The shCtrl or PBS control values were set as 100%. Error bars, s.e.m. 

**n = 10 each for (a) and n = 3 each for (b). **P < 0.05, **P < 0.01. NS, not statistically significant (P > 0.05).

c. AKT activation status in BCAT1- or MSI2-knockdown K562 cells. K562 cells treated with shCtrl, shBCAT1 or shMSI2 were analysed by western blotting for pAkt (at Thr308 or Ser473), total AKT, hBCAT1, hMSI2 and HSP90 levels.

d. Effect of α-ketoglutarate supplementation on the colony-forming ability of BCAT1-knockdown cells. K562 cells transduced with shCtrl (−) or shBCAT1-d (+) were plated in triplicate with or without 1 mM dimethyl-α-ketoglutarate (KG) and/or 4 mM BCAAs as indicated. n = 3 technical replicates. Error bars, s.e.m. *P < 0.05, **P < 0.01. NS, not statistically significant (P > 0.05).
Extended Data Figure 9  |  MSII and BCAT1 expression in human cancer. a, Microarray data analysis of MSII expression in human CML. Gene expression data of chronic (grey, n = 57), accelerated (pink, n = 15) and blast crisis (blue, n = 41) phase patients were retrieved from the GEO database (accession number GSE4170). Bar, the normalized expression value in each specimen. b, Co-expression analysis of the BCAT1 and MSII genes in human cancer. Pearson correlation coefficients were used to evaluate the extent of co-expression patterns. c, Schematic of the human BCAT1 transcript. Bars, the putative MBEs (r(G/A)U1–3AGU). Forty MBEs were identified within the 3′ UTR of BCAT1. CDS, coding sequence for hBCAT1 protein. d, K562 cells infected with lentiviral shRNA against MSII (shMSII) or shCtrl (−) were analysed by western blotting for pS6 kinase (at Thr389), total S6K, hMSII and HSP90 levels. Note that MSII knockdown reduced the levels of BCAT1 protein and pS6K.