Autophagy regulates fatty acid availability for oxidative phosphorylation through mitochondria-endoplasmic reticulum contact sites

Claudie Bosc, Nicolas Broin, Marjorie Fanjul, Estelle Saland, Thomas Farge, Charly Coudry, Aurélie Batut, Rawand Masoud, Clément Larrue, Sarah Skuli, Nicolas Espagnolle, Jean-Christophe Pagès, Alice Carrier, Frédéric Bost, Justine Bertrand-Michei, Jérôme Tamburini, Christian Récher, Sarah Bertoli, Véronique Mansat-De Mas, Stéphane Manenti, Jean-Emmanuel Sarry, Carine Joffre

Autophagy has been associated with oncogenesis with one of its emerging key functions being its contribution to the metabolism of tumors. Therefore, deciphering the mechanisms of how autophagy supports tumor cell metabolism is essential. Here, we demonstrate that the inhibition of autophagy induces an accumulation of lipid droplets (LD) due to a decrease in fatty acid β-oxidation, that leads to a reduction of oxidative phosphorylation (OxPHOS) in acute myeloid leukemia (AML), but not in normal cells. Thus, the autophagic process participates in lipid catabolism that supports OxPHOS in AML cells. Interestingly, the inhibition of OxPHOS leads to LD accumulation with the concomitant inhibition of autophagy. Mechanistically, we show that the disruption of mitochondria-endoplasmic reticulum (ER) contact sites (MERCs) phenocopies OxPHOS inhibition. Altogether, our data establish that mitochondria, through the regulation of MERCs, controls autophagy that, in turn finely tunes lipid degradation to fuel OxPHOS supporting proliferation and growth in leukemia.
Autophagy is a dynamic catabolic process in which cytoplasmic components, including proteins and organelles, are sequestered into specific intracellular vesicles, called autophagosomes, before to be delivered to lysosomes for degradation\(^1\). However, autophagy is not only a sink since degradation products are reused by the cell to sustain metabolism and allow cell survival. Autophagy is a sensor of the metabolic state and allows cells to adapt their demands to poor growth environments, and is considered as a crucial survival mechanism\(^2\). In physiological conditions, autophagy is for instance required to provide amino acids to the starving neonates after birth\(^3\) or to maintain circulating glucose levels in fasting adult mice\(^4\).

Autophagy has also been critically implicated in tumorigenesis. This catabolic process was first shown to favor cancer progression\(^5\)–\(^7\), at least in part through its capacity to support the proliferation and survival\(^7\) of circulating glucose levels in fasting adult mice\(^4\).

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**Results**

Autophagy participates to lipid catabolism to support OxPHOS. Whether FFA\(^\text{s}\) might represent a critical substrate to fuel OxPHOS in AML cells compared to their normal healthy counterpart cells (hereafter normal hematopoietic cells) is still not known. To address this question, AML blasts (from peripheral blood; Supplementary Table 1) and normal hematopoietic cells (peripheral blood mononuclear cells (PBMC) and CD34\(^+\) cells; Supplementary Table 2) were first treated with a low concentration of etomoxir (Et\(\text{x}\), 3 \(\mu\)M) to prevent the entry of FFAs into mitochondria by blocking the activity of carnitine palmitoyl transferase 1, and oxygen consumption rate (OCR) was evaluated by Seahorse analysis. We found that the OCR (Fig. 1a, Supplementary Fig. 1a, b) and ATP production linked to respiration (Supplementary Fig. 1d) were significantly decreased up to 30–50\% in AML cells from two cell lines (MOLM14 and U937) and in primary patient samples compared to respective control AML cells. Conversely, basal OCR and ATP-linked OCR in the presence of Et\(\text{x}\) were only slightly decreased in primary normal hematopoietic cells (Fig. 1a, Supplementary Fig. 1c, d). Therefore, FFAs represent a minor substrate in normal hematopoietic cells, but are an important carbon source that fuels the TCA cycle and oxidative phosphorylation in AML cells.

We next addressed the origin of FFAs in AML. Autophagy can generate FFAs via the degradation of LD\(^5\)–\(^6\) and has been implicated in tumor lipid homeostasis\(^7\)–\(^9\). As autophagy has also been shown to be required for AML cell proliferation\(^9\), we hypothesized that autophagy also contributes to lipid catabolism to support TCA cycle and mitochondrial energetic metabolism in AML cells. To test this hypothesis, two human AML cell lines were first treated with a well-known pharmacologic inhibitor of autophagosome formation, 3-methyladenine (3-MA), and lipid content was analyzed by flow cytometry using the BODIPY 493/503 probe. Treatment with 3-MA revealed a significant increase in fluorescence intensity as compared to control conditions, indicating an accumulation of lipids after inhibition of autophagy (Supplementary Fig. 1e). Consistent with these results, microscopy studies revealed a cytosolic accumulation of lipid in structures resembling LD in AML cells treated with 3-MA, with no change in lipid content in normal hematopoietic cells (Fig. 1b–d, Supplementary Fig. 1f). This suggests that the involvement of autophagy in lipid metabolism of normal hematopoietic cells is negligible compared to AML cells. To rule out 3-MA off-target effects, we investigated the consequences of short-term and long-term silencing of the key autophagy proteins Beclin1 and ATG12 on lipid accumulation. Similar to 3-MA treatment, the number and area of BODIPY 493/503-positive structures increased upon genetic inhibition of autophagy in AML cells (Fig. 1e–g, Supplementary Fig. 1g). Of note, no change in lipid content of normal hematopoietic cells was significantly observed (Supplementary Fig. 1h, i). This demonstrates that autophagy regulates lipid content in AML cells. Furthermore, specific acid lipase lysosomal inhibitor Lalistat2 also promoted the accumulation of BODIPY 493/503-positive structures in AML cells (Supplementary Fig. 1j, k). To further characterize these intracellular organelles, we quantified content in triglycerides, one of the major storage lipids sequestered in LD. We observed a significant increase in triglyceride level in autophagy-incompetent cells (Fig. 1h). These results combined with the intracellular punctuated BODIPY staining indicate that inhibition of autophagy led to the accumulation of triglycerides in LD in AML cells. Altogether these experiments strongly suggest that autophagy is involved in lipid catabolism in AML cells to generate FFAs necessary for mitochondrial activity, and that this process is not observed in normal hematopoietic cells, where lipids are not a major respiratory source.

Since fatty acid oxidation (FAO) is the biochemical process responsible for FA degradation and indicative of the level of FFAs available to cells, we next analyzed FAO in AML cells. As expected, the inhibition of autophagosome formation by an shRNA directed against ATG12 (Fig. 1i) or by 3-MA (Supplementary Fig. 1l) reduced the rate of FAO. Thus, autophagy of LD (i.e., lipophagy) contributes at least in part to the release, and availability of FFAs from triglyceride hydrolysis in AML cells. We therefore studied whether the FFAs released by autophagy and utilized by mitochondria represent an important respiratory substrate that participates in ATP production by OxPHOS. To do so, we assessed OCR and mitochondrial ATP production in cells with inhibited autophagy. This approach revealed that both basal OCR and mitochondrial ATP production-linked OCR were significantly decreased, when autophagy was inhibited either with 3-MA (Fig. 1j, Supplementary Fig. 1m, n) or with a small
interfering RNA (siRNA) targeting Beclin1 in AML cells (Fig. 1k, Supplementary Fig. 1o, p). Conversely, OCR and mitochondrial ATP production were slightly decreased in normal hematopoietic cells (Supplementary Fig. 1q, r). In summary, these results indicate that AML cells in part rely on autophagy for energy metabolism by supplying FFAs to fuel oxidative phosphorylation.

**Inhibition of OxPHOS affects lipid metabolism.** Given that our findings established that FFAs are essential for mitochondrial activity, we next wondered whether OxPHOS could in turn regulate the lipid metabolism. Transcriptomic analysis of AML cells treated with the mitochondrial electron transfer chain (ETC) complex I inhibitor metformin\(^{18}\) (Supplementary Fig. 2a) showed a downregulation of genes regulating metabolic processes (Fig. 2a,
Fig. 2 Inhibition of OxPHOS affects lipid metabolism. a Volcano plots displaying fold change versus adjusted p-values of MOLM14 (left) and U937 (right) cells treated with 10 mM of metformin (Met) for 24 h (p-value < 0.05, absolute log2 fold change > 0.5, unpaired t-test). b Venn diagram representing overlap between MOLM14 and U937 downregulated (left) and upregulated (right) genes. Enrichment analysis of Gene Ontology (GO) classification for common downregulated (left) and upregulated (right) genes by Genomatics software analysis (Fisher’s exact test). c MOLM14 cells treated with Met (10 mM) for 24 h were fixed and processed for transmission electron microscopy analysis. Representative electron microscopy pictures from two independent experiments are shown. Arrows indicate lipid droplets. Scale bar: 2 μm. d MOLM14 cells were treated with Met or with antimycin A (AA) and processed for triglycerides content analysis. (n = 3, unpaired t-test). e MOLM14 cells were treated with Met or with AA, fixed and stained for Bodipy 493/503 and DAPI. Representative confocal pictures from three independent experiments are shown. Scale bar: 2 μm. f g MOLM14 (n = 3) and U937 (n = 3) AML cell lines, primary AML patient cells (n = 14) and primary normal hematopoietic cells (PBMC n = 9; CD34+ n = 4) were treated with Met or with AA for 48 h, fixed, and stained for Bodipy 493/503 and DAPI. Histograms show the number (f) or the area (g) of Bodipy 493/503 dots per cell (unpaired t-test or paired t-test for patients’ samples). Data are means ± s.e.m.
mitochondrial respiratory chain inhibition led to an accumulation of LD in AML cells and had no impact on normal hematopoietic cells (Fig. 2e–g, Supplementary Fig. 2g). Of note, similar results were obtained with cells grown in lipid-free serum medium, suggesting that the increase in LD resulted from the accumulation of both extracellular and endogenous lipids (Supplementary Fig. 2h, i).

Next, we examined whether this accumulation was due to an increased lipogenesis or a decrease in degradation. As previously reported29, metformin strongly reduced de novo lipogenesis (Supplementary Fig. 2j). Importantly, our transcriptomic analysis did not reveal any increase in gene expression, and signatures related to the biogenesis or the trafficking of LD, to the synthesis of triglycerides, or to transcription factors regulating lipogenesis (Supplementary Tables 3–5). In addition, no change in the expression level of proteins implicated in LD formation (i.e., ADRP, Supplementary Fig. 2k), or modulation of the subcellular localization of the transcription factor SREBP1/2 were observed upon metformin or AA treatment (Supplementary Fig. 2l). Moreover, both metformin and AA markedly reduced FAO (Supplementary Fig. 2m), suggesting that the inhibition of OxPHOS also prevented the lipid degradation. Collectively, these data indicate that mitochondrial OxPHOS is directly linked to the lipid metabolism in AML cells by controlling the degradation of LD and the availability of respiratory substrates.

Inhibition of OxPHOS reduces autophagic flux. Based on above-described results, we next sought to determine whether mitochondrial function might regulate lipid degradation via the control of the autophagy process. To address this possibility, we evaluated if inhibiting the mitochondrial ETC could modulate autophagy. To measure the autophagic flux, the conversion of LC3B-I to LC3B-II that reflects the number of autophagosomes30 was assessed by immunoblotting in presence or absence of chloroquine (chloro), an inhibitor of lysosomal degradation. These experiments revealed that LC3B-II accumulation was significantly (>50% after 48 h) decreased in metformin- or AA-treated AML cells from cell lines and primary patient specimens (Fig. 3a–f, Supplementary Fig. 3a–c), but not in normal hematopoietic cells (Fig. 3e, f). We confirmed the impact of ETC on autophagy with immunofluorescence studies of endogenous LC3B. The number of autophagosomes per cell was significantly decreased upon metformin treatment compared to controls in all AML cells, without modification in number of autophagosomes detected in healthy cells (Fig. 3g–i, Supplementary Fig. 3d–f). Furthermore, flow cytometry analysis of autophagic vacuoles using Cyto-ID assay fully confirmed this observation in AML cells (Supplementary Fig. 3g). Altogether these approaches consistently support the contention that mitochondrial ETC and OxPHOS activity positively regulate autophagy in AML cells, but not necessarily in normal hematopoietic cells.

OxPHOS regulates MERCs number and function. These above-mentioned results demonstrate that the capacity of mitochondria to regulate the lipid availability through autophagy seems unique to tumor cells. Therefore, we next investigated how mitochondria mechanically control autophagy in AML cells. Electron microscopy analysis of AML cell lines showed that ~20% of the mitochondria were in close proximity to the ER (Fig. 4a, b, Supplementary Fig. 4a, b). These inter-organella contacts between mitochondria and the ER called MERCs are now recognized as essential regulators of fundamental cellular processes, including autophagy31, bioenergetics32, and as key players in oncogenesis33,34. Interestingly, metformin markedly reduced the number of these MERCs (Fig. 4a, b, Supplementary Fig. 4a, b) without affecting the length (MOLM14: 204 nm ± 22; MOLM14 + Met: 228 nm ± 20; U937: 361 nm ± 36; U937 + Met: 253 nm ± 27), the number or mass of mitochondria (Supplementary Fig. 4c, d). Expectedly, since one of the key functions of MERCs is to regulate calcium homeostasis, both metformin and AA reduced the mitochondrial calcium content. This thus suggests a MERCs-dependent diminished Ca2+ transfer from ER to mitochondria (Supplementary Fig. 4e). In addition, MERCs support autophagy by the recruitment of protein–protein complexes implicated in the autophagosome formation34,35. To next validate the role of MERCs in autophagosome formation in AML cells, we developed a protocol based on Wieckowski et al. subcellular fractionation procedure36. We fractionated extracts of AML cells and characterized the MERCs fraction by the presence of FALC4 (Fig. 4c).

Moreover, Mtfn2-depleted cells displayed a significantly decreased the number of autophagosomes (Fig. 5d, e) and the autophagic flux (Supplementary Fig. 5d, e) in AML cells. In addition, the silencing of an ER protein, IP3R1, rather than a mitochondrial protein, reduced also the autophagic flux (Supplementary Fig. 5f). As
expected from our hypothesis, fluorescent microscopy analysis showed that both Mtfn2 or VDAC1 depletion led to an accumulation of LD compared to control cells (Fig. 5f, g). Accordingly, electron microscopy analysis showed that Mtfn2- or VDAC1-silenced AML cells accumulated LD (Fig. 5a, Supplementary Fig. 5a). Moreover, since the expression of FASN, ACLY, and ADRP was not modified and no change in the subcellular localization of the transcription factor SREBP1/2 was observed upon Mtfn2 depletion (Supplementary Fig. 5g, h), LD biogenesis is likely not affected with MERCs inhibition. All of these results phenocopied the effects observed upon metformin treatment (Fig. 2c, Supplementary Fig. 2k, l). Collectively, these data indicate that autophagy inhibition observed upon mitochondrial respiration inhibition was due to a reduction in the number of MERCs and is consistent with mitochondria regulating autophagy through mitochondria–ER contact sites in AML cells. Similar to autophagy inhibition (Fig. 1j, k, Supplementary Fig. 1m-p), the decrease of MERCs upon VDAC1 or Mtfn2 depletion resulted in a decreased OCR (Fig. 5h, Supplementary Fig. 5i, j) and mitochondrial ATP production-linked OCR.
compared to control cells (Supplementary Fig. 5k). Furthermore, when autophagy was inhibited (siBeclin1) or MERCs were disrupted (siMtfn2), mitochondria of AML cells pulsed with a fluorescent fatty acid lipid (RC12) exhibited less overlap with this lipid due to the accumulation of RC12 into the cytoplasm (Fig. 5i, j). These results indicate that lipids upon autophagy inhibition or MERCs disruption were not utilized and oxidized by mitochondria. Furthermore, the addition of exogenous fatty acids upon autophagy inhibition or MERCs disruption restored mitochondrial respiration (Fig. 5k). Altogether, these results suggest that MERCs through autophagy regulation control lipid catabolism that supports mitochondrial OxPHOS in AML cells. Since ETC inhibitors reduced MERCs number and functions (Fig. 4, Supplementary Fig. 4), we confirmed that autophagy inhibition and subsequent accumulation of LD in metformin-treated cells are due to the loss of MERCs by using an organelle linker, as performed by Cserdás G. et al. Expression of this organelle linker in MOLM14 cells (OMM-ER) reactivated calcium flux from ER into mitochondria and therefore restored MERCs upon metformin treatment (Supplementary Fig. 5l). This prevented autophagy inhibition and lipid accumulation induced by metformin treatment (Fig. 5l–o). Altogether, these data highlight the role of MERCs formation regulated by mitochondria activity in autophagosome formation to promote lipid degradation and mitochondrial OxPHOS in AML cells.

**MERCs support the dialog between autophagy and OxPHOS.** Finally, to test the functional relevance of the role of autophagy-dependent MERCs formation, we monitored the proliferation of VDAC1-depleted cells in vitro and in vivo, or we daily treated NOD-SCID-gamma (NSG) immunodeficient mice engrafted with AML MOLM14 cells, with an oral potent ETC complex I inhibitor, the IACS-010759. We first validated that this ETC complex I inhibitor phenocopied in vitro the results observed with other ETC inhibitors metformin or AA on lipid metabolism and autophagy. As expected, IACS-010759-treated cells accumulated LD (Supplementary Fig. 6a, b) and displayed a reduction of the autophagic flux (Supplementary Fig. 6c–f). These experiments showed that VDAC1 depletion or IACS-010759 treatment significantly affected cell proliferation in vitro (Supplementary Fig. 6g, h) and tumor growth in vivo (Fig. 6a, c). Mice engrafted with shVDAC1 AML cells or treated with IACS-010759 displayed a marked reduction of the total tumor cell burden and a significant increase in overall survival compared to mice injected
with control cells (Fig. 6b, d). Furthermore, we observed an increase of lipids (Fig. 6e–g), a decrease in autophagy (Fig. 6h) along with a reduction of MERCs (Fig. 6i) in human AML cells from mice treated with IACS-010759, confirming that the observed impact of IACS-010759 in vivo is due to MERCs disruption with subsequent autophagy inhibition and lipid accumulation. Thus, proliferation and tumorigenicity of AML cells are linked to their ability to produce FFAs via autophagy to ensure mitochondria function that controls in turn autophagy through MERCs formation (Fig. 6j).
Fig. 5 MERCs regulate lipophagy to sustain OxPHOS. a MOLM14 cells transfected with siRNA control (Ctrl) or mitofusin2 (Mtfn2) were processed for electron microscopy analysis. Electron microscopy pictures from one experiment are shown. Scale bar: 1 µm. b Histograms represent the % of mitochondria in contact with ER. Each dot corresponding to one cell. c Images of proximity ligation assay between VDAC1 and IP3R1 from MOLM14 transfected with Ctrl or Mtfn2 siRNAs (n = 3). Scale bar: 10 µm. Numbers represent the % of dots in cells transfected with siRNA Ctrl. d, e MOLM14 cells were transfected with Ctrl or VDAC1 shRNAs (n = 4) or transfected with Ctrl or Mtfn2 siRNAs (n = 6), and stained for LC3B and DAPI. Confocal sections (at least four independent experiments) are shown. Scale bar: 10 µm (d). Histograms represent the number of LC3B puncta per cell (e) (unpaired t-test). f, g MOLM14 cells transfected with Ctrl or VDAC1 shRNAs (n = 3) or transfected with Ctrl or Mtfn2 siRNAs (n = 6) were stained for Bodipy and DAPI. Scale bar: 10 µm, (f). Histograms show the number or the area of Bodipy dots per cell (g) (unpaired t-test). h Measurement of basal oxygen consumption rate (OCR) in MOLM14 cells transfected with Ctrl or VDAC1 shRNAs (n = 6) or transfected with Ctrl or Mtfn2 siRNAs (n = 6, unpaired t-test). i, j MOLM14 cells transfected with Ctrl or Beclin1 (Bec) or Mtfn2 siRNAs and incubated with RC12, were stained for TOMM20 and confocal Z-stacks were acquired (three independent experiments). Scale bar: 10 µm (i). Fraction of RC12 overlapping TOMM20 staining (j). k Measurement of OCR in MOLM14 cells transfected with Ctrl (n = 8), Bec (n = 7), or Mtfn2 siRNAs (n = 8) ± Oleate-BSA. l-o MOLM14 cells were transfected with the mitochondria-ER organelle linker (OMM-ER) and its control (OMM), treated ± mitofusin and subjected to Western blot analysis for actin and LC3B (l, m) or stained for Bodipy and DAPI (n, o) (three independent experiments). Scale bar: 10 µm. Histograms represent the number of LC3B puncta (m) or the area of Bodipy dots per cell (o) (unpaired t-test). Data are means ± s.e.m.

to support OxPHOS, and the role of lipids as an essential substrate for AML cell proliferation both in vitro and in vivo (Fig. 6).

Discussion

Autophagy is frequently activated in cancer and largely associated with metabolic reprogramming and oncogenesis. Our findings show that autophagy occurring at a specific subcellular location, namely the interconnection between mitochondria and the ER, supplies FFAs to maintain mitochondrial energy metabolism that enables cell proliferation in vitro and in vivo. Furthermore, we found that mitochondrial respiratory chain activity supports autophagy through the regulation of MERCs formation. Altogether these data support a model depicting a bidirectional relationship between autophagy and mitochondria metabolism, in which mitochondria regulates its supply of FFAs by regulating autophagosome formation at MERCs. This relationship allows for the physical and functional integration of LD degradation with the supply of FFAs to fuel the TCA cycle in adjacent mitochondria, and supports the notion that lipid transfer proteins could be localized at MERCs (Fig. 6).

Autophagy could degrade LD and release FFAs that are then oxidized via mitochondrial FAO. ETC inhibitors including metformin are known to regulate autophagy. However, while metformin is mainly described as an autophagy inducer through its agonist action on AMPK[41,42], we report here alongside with two studies[43,44], the unexpected finding that OxPHOS inhibitors such as metformin can interfere with and inhibit autophagosome formation. Moreover, as opposed to what it was shown in adipocytes and hepatocytes[45,46], we showed that metformin inhibits lipid degradation and FAO in AML cells. This occurred at less extend in normal hematopoietic cells.

Cumulatively, our results indicate that the inhibition of autophagy and the subsequent accumulation of LD observed upon ETC inhibition are a consequence of MERCs disruption. Upon ETC inhibition, MERCs alteration is an early event (6 h) before the reduction of autophagy (24 h) and the accumulation of LD at later stage (48 h). In addition, restoring MERCs formation and calcium uptake by the mitochondria using the mAKAP1-mrFP-yUBC6 (i.e., OMM-ER) organelle linker[39] prevented metformin-induced autophagy inhibition and LD accumulation. MERCs have been previously reported as an important site of autophagosome formation in cancer cells[34, fibroblasts[35], and in epithelial cells[47]. However, the role of MERCs in autophagosome formation was not yet described in AML cells. Then, the constitutive presence of key molecules involved in the early events of autophagic flux (Vps34 and ATG16L1, two proteins located on the isolation membrane during the first step of autophagosomes formation) in purified MERCs confirms their crucial roles in autophagosome formation and in AML autophagy.

Cancer cells usually exhibit an exacerbated metabolism compared to their normal counterpart. Even if this metabolic reprogramming, known as the Warburg effect, originally consisted to use the glycolysis to produce energy, a vast majority of cancer cells rely rather on mitochondrial oxidative metabolism[48]. Several solid and blood cancers have dependency on OxPHOS and/or FAO pathways either in steady state[28,49,50] and in metastasis[51,52], but also upon treatments[17,18]. This suggest that understanding better the contribution of respiratory sources, such as FFAs to mitochondrial OxPHOS[44] will be crucial to deepen fight against cancer, drug resistance, and relapse. Therefore, our study identified the interplay between mitochondrial metabolism and autophagy as a critical regulator in AML cell proliferation, and could represent a potential therapeutic target for AML patients. This should be further explored in other cancers that rely on mitochondrial metabolism[53,54].

Methods

AML cell lines. The human myeloid leukemia cell lines, MOLM14 and U937 were purchased from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Leibniz, Germany). MOLM14 shATG12 were previously generated in our laboratory. MOLM14 CRISPR CTL or AMPK-KO were a gift from Prof. Jérome Tamburini (University of Geneva, Switzerland). Cells were grown in minimum essential medium-a medium with Glutamax ( Gibco, Life Technologies) supplemented with 10% fetal calf serum (Sigma).

Primary AML cells and normal hematopoietic cells. Primary AML patient cells from peripheral blood have been collected during routine diagnostic procedures at the Toulouse University Hospital, after informed consent and stored at the HIMIP collection (BB-0033-00060). According to the French law, HIMIP collection has been declared to the Ministry of Higher Education and Research (DC 2008-307 collection) and obtained a transfer agreement (AC 2008-129) after approval by the “Comité de Protection des Personnes Sud-Ouest et Outremer II” (ethical committee). PBMC were obtained from blood samples of healthy donors (Établissement Français du Sang, EFS, Toulouse, France). CD34 positive cells were obtained after sorting from umbilical cord blood samples (Établissement Français du Sang, EFS, Besançon, France). Normal hematopoietic cells (PBMC and CD34+) were obtained from EFS that is a governmental agency collecting and delivering blood products, all procedures in use at EFS are defined by the Law. For samples aimed at research use a personal “informed consent” form is signed at the time of collection. This form was validated by the “Agence de la Biomédecine” the body that, in France, rules all type of samples of human origin, on ethical and practical aspects, for research or clinical applications. Clinical and biological annotations of the samples have been declared to the CNIL (Comité National Informatique et Libertés, i.e., Data processing and Liberties National Committee). Briefly, mono-nuclear cells were separated by Ficoll–Hypaque density gradient centrifugation and incubated in RBC lysis buffer (ammonium chloride solution) to remove red blood cells. CD34+ cells isolation was performed using manufacturer’s instructions (EasySepTM, STEMCELL). Primary AML, PBMC, and CD34+ samples were
Antibodies and reagents. The following antibodies from Cell Signaling Technology were used: rabbit antibodies against LC3B (#2775), ATG12 (#4180), FASN (#3180), Beclin1 (#3738), and mouse antibodies against HSP90 (#4874). Mouse antibodies from Santa Cruz Biotechnology against IP3R1 (sc-271197) and E2F1 (KH95), from Millipore against actin, rabbit antibody from Abcam against VDAC1 (ab15865), from PROGEN against ADRP (610102), from R&D System against FABP4 (AF3150), from Atlas Antibodies against ACLY (HPA022434), from Novus Biologicals against SREBP1 (NB600-582), from Invitrogen against SREBP2 (PA1-338), and from GeneTex against TOMM20 (GTX133756) were also used. Secondary antibodies labeled with horseradish peroxidase were purchased from Pro- mega and those coupled with fluorophores were purchased from Invitrogen. Rabbit anti-LC3B from MBL (#PM036) was used for immunofluorescence analysis. For flow cytometry studies, the antibodies anti-hCD45-APCH7 (BD Biosciences; 641417), CD33-PE (BD Biosciences; 555450), Annexin-V-V500 (BD Biosciences; 561501), and BD Via-ProbeTM (BD Pharmingen) were used. Baflomycin (20 nM) and 3-MA (5 mM) were purchased from InvivoGen. Chloroquine (20 μM), metformin (10 mM), antimycin (10 μM), and lalistat2 (20 μM) were obtained from Sigma Aldrich. BodipyTM 493/503 probe was purchased from ThermoFisherScientific. Etx (3 μM, HY-50202A) was purchased from Medchem. EBSS (24010-43, Gibco) was used to induce autophagy.

Subcellular fractionation. Buffer A: 20 mM Hepes pH7.4, 10 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 20% glycerol, 1 mM DTT, 0.1% NP-40, and protease inhibitors.

Buffer B: 20 mM Hepes pH7.4, 500 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 20% glycerol, 1 mM DTT, 0.1% NP-40, and protease inhibitors.

maintained in Iscove’s Modified Dulbecco’s Medium supplemented with 20% fetal calf serum.
Cells were washed with ice-cold phosphate-buffered saline (PBS) pH 7.4. After centrifugation, cell pellets were resuspended in 250 μL of ice-cold buffer A and kept for 10 min in ice. A total of 20 μL of the lysate were removed as “total lysate” and the nuclei were pelleted by centrifugation at 500 × g for 5 min at 4 °C. The supernatant was removed as the “cytosolic fraction” and the pellet of nuclei was gently washed with 350 μL of buffer A and centrifuged at 500 × g for 5 min. The supernatants were discarded. The nuclear pellets were resuspended in 100 μL of hypotonic buffer B and allowed to swell on ice for 30 min. The extract was separated by centrifugation at 21,000 × g for 15 min at 4 °C. The supernatant was collected and designated as “nuclear fraction.” All fractions were resuspended in Laemmli sample buffer and “nuclear fractions” and “total lysate” that contained DNA were sonicated.

Isolation of mitochondria-associated membranes. MERCs fractions were isolated according to the Nature Protocols from Wieckowski et al.36. The cell number and the cell lysis method were adapted for AML cells. Briefly, 2.3 billion of MOLM14 cells were washed with PBS (Ca2+ and Mg2+) and centrifuged at 600 × g for 5 min at 4 °C, and the cell lysis method were adapted for AML cells. Brieﬂy, 2.3 billion of MOLM14 cells were washed with PBS (Ca2+ and Mg2+) and centrifuged at 600 × g for 5 min at 4 °C. The supernatant was discarded. The cell lysis method were adapted for AML cells. Brieﬂy, 2.3 billion of MOLM14 cells were washed with PBS (Ca2+ and Mg2+) and centrifuged at 600 × g for 5 min at 4 °C. After two centrifugations, the pellet was discarded. The supernatant was collected and centrifuged at 7000 × g for 10 min at 4 °C to separate crude mitochondria (pellet) from microsome and ER fractions (supernatant). The crude mitochondrial fraction was suspended in 1 mL of buffer 1 (225 mM mannitol, 75 mM sucrose, 0.1 mM EGTA, and 30 mM Tris HCl pH 7.4) and disrupted using a nitrogen cavitation chamber (PARR Instrument, 7 min at 35 psi). Nuclei and unbroken cells were pelleted by centrifugation at 600 × g for 5 min at 4 °C. After two centrifugations, the pellet was discarded. The supernatant was collected and centrifuged at 7000 × g for 10 min at 4 °C to separate crude mitochondria (pellet) from microsome and ER fractions (supernatant). The crude mitochondrial fraction was suspended in 1 mL of buffer 2 (225 mM mannitol, 5 mM HEPES, and 0.5 mM MgCl2) for 5 min at 4 °C. Mitochondrial suspension was centrifuged at 7000 × g for 10 min at 4 °C, and the supernatant was discarded. Mitochondrial pellet was suspended into 1 mL of buffer 2 and centrifuged at 10,000 × g for 10 min at 4 °C. The crude mitochondrial pellet was suspended into 2 mL of MRB buffer (250 mM mannitol, 5 mM HEPES, and 0.5 mM EGTA) with 2 μl of Annexin-V-V500 and 2 μl of CD33-PE (BD Biosciences; 555450) for 20 min, and cells were washed in PBS and resuspended in Annexin-V binding buffer (BD biosciences; 556454) plus 2 μl of Annexin-V-V500 (BD Biosciences; 561501). Absolute cell numbers of viable human blasts (CD45+/Annexin-V-) were quantified using high resolution fluorescence microscopy. Images were taken with a Zeiss LSM 880 FAST Airyscan using a 63× Plan-Apochromat objective with 1.4 aperture under immersion oil. The Manders 2 (M2) coefficient, or fraction of RC12 signal overlapping mitochondrial network, was determined from Z-stack projections of RC12 and mitochondrial network using the Fiji JACoP plugin.

Immunofluorescence analysis. For LC3B staining, cells were seeded onto glass slides (Fisher Scientific) coated with 0.01% poly-L-lysine (Sigma), then fixed in 4% formaldehyde for 8 min. After PBS washes, cells were incubated in 0.1% saponin containing 3% BSA for 30 min and then incubated with anti-LC3B antibodies (MBL, 1:700) for 45 min. Cells were then washed before incubation with an anti-rabbit Alexa-488 secondary antibody (Invitrogen) for 30 min, followed by PBS and distilled H2O washes and mounting in ProLong™ Gold antifade medium with DAPI (4′-diamidino-2-phenylindole, Invitrogen). Images were acquired using a confocal Zeiss LSM 780. For quantification, fields were chosen arbitrarily based on DAPI staining, and the number of LC3B dots per cell of at least 100 cells per independent experiment was determined with ImageJ software.

Lipid chase. For lipid chase experiments, AML cells were labeled with 1 μM of the fluorescent fatty acid BODIPY 558/568 C12 (RC12-Thermo Fisher Scientific) overnight in complete culture medium. After two washes, AML cells were seeded on coverslips, fixed, and stained for mitochondrial network with TOMM20 (Genetex, 133736) like for LC3B staining. For imaging of the mitochondrial network and RC12 distribution, 6–8 μm Z-stacks at 18.1 μm were used. Fluorescence signals were analyzed using high resolution fluorescence microscopy. Images were taken with a Zeiss LSM 880 FAST Airyscan using a 63× Plan-Apochromat objective with 1.4 aperture under immersion oil. The Manders 2 (M2) coefficient, or fraction of RC12 signal overlapping mitochondrial network, was determined from Z-stack projections of RC12 and mitochondrial network using the Fiji JACoP plugin.

Duolink PLA. Duolink II PLA (Sigma) enables the detection of protein interactions (~40 nm) as an individual fluorescent dot by microscopy. Cells were fixed 10 min with 4% paraformaldehyde and permeabilized for 15 min with PBS 0.1% Triton X100. The proximity ligations were performed according to the manufacturer’s protocol. Preparations were mounted and analyzed similarly to immunofluorescence.

Flow cytometry (FACS) analysis. Flow cytometry experiments were performed on CytoFLEX flow cytometer (Beckman Coulter) instrument. After harvesting, suspensions of murine bone marrow and spleen were stained with 2 μl of CD45-APC7 (BD Biosciences; 641417), 2 μl of CD33-PE (BD Biosciences; 555450) for 20 min, and cells were washed in PBS and resuspended in Annexin-V binding buffer (BD biosciences; 556454) plus 2 μl of Annexin-V-V500 (BD Biosciences; 561501). Absolute cell numbers of viable human blasts (CD45+/CD33+/Annexin-V-) were quantified using CountBright™ (Invitrogen). FACS analysis was also used to determine the mitochondrial mass of AML cells. Cells were washed with PBS and stained with MitoTracker® Green FM (MTG, 1/10,000) for 20 min at 37 °C. Cells were then washed and resuspended in Annexin-V binding buffer (BD biosciences; 556454) plus 2 μl of Annexin-V-V500 (BD Biosciences; 561501). Cell lipid content was measured by flow cytometry using the Bodipy 493/503 probe. Briefly, cells were washed, incubated with Bodipy (0.5 μg/mL) for 30 min at 37 °C, then washed and resuspended with Annexin-V binding buffer (BD biosciences; 556454) and stained with Annexin-V-AP (BD biosciences; 550474) to exclude apoptotic cells. Data were analyzed with FlowJo v10 software (Tree Star Inc., Ashland, OR, USA).

Cyto-ID®. Autophagic flux was assessed using the Cyto-ID®-based procedure according to the manufacturer’s instructions (Enzo Life Sciences, Switzerland). The fluorescence of the Cyto-ID® dye incorporated into the different AML cells was analyzed by a fluorescence-activated cell sorter on a Maccsant (Miltenyi Biotec, Paris, France) flow cytometer.
Detection of lipid droplets by microscopy analysis. Lipid droplets were stained using the Bodipy 493/503 probe. After cell plating on glass coverslips and fixation (see above), cells were washed and incubated with 1 µg/mL of Bodipy 493/503 diluted in 150 mM NaCl for 10 min at room temperature. Cells were then washed and slides mounted with ProLongTM Gold antifade medium with DAPI (Invitro- gen). The number and the area of Bodipy dots per cell of at least 100 cells per independent experiment were determined with Image J software.

Transcriptional and ultrastructural analysis. Cell pellets were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 4 h. They were then postfixed with 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 h, dehydrated in graded ethanol, and embedded in epon-araldite mixture. Ultrathin sections were stained with uranyl acetate and Reynolds’s lead citrate, and examined in a Hitachi HT7700 transmission electron microscope (Hitachi-High Technolo- gies, Tokyo, Japan) at 80 kV with acquisition performed on a CCD AMT XR41 camera at the CMEAB (Toulouse) or in an FEI Tecnai G2 at 200 kV with acquisition performed on a Veleta camera (Olympus, Japan) at the PICSL-FBI core facility (IBDM, AMU-Marseille).

Plasmids and lentiviral infection. Control Pool Non-Targeting #2 (Ref D-001206-14-20; UAAGGCUAUGAAGAG) and Plasmids and lentiviral infection. OMM-ER and OMM, respectively) were a gift from Prof. Gyo czky (Thomas Jefferson University, Philadelphia). These sequences were added into an inducible lentiviral vector: Pinducer21. After incubation, FAO was measured by 14CO2 trapped in 300 µL of benzethonium chloride, as described above. IACS-10759 was kindly provided by Dr. M. Konoplova and J. Marxaleur. IACS-10759 was solubilized in water containing 0.5% methylcellulose before adminis- tration to mice. IACS-10759 was administered subcutaneously at a single dose of 4 mg/kg, at 1-week intervals. Mice were housed in sterile conditions using HEPA-filtered micro- isolators, and fed with irradiated food and sterile water. Eight-week-old mice were subcutaneously treated with busulfan (20 mg/kg) 24 h before injection of AML cells. AML cells (MOLM14, MOLM14 shARN control, or MOLM14 shARN VDAC1) were transduced cells with 1 µg/mL puromycin. All shRNA experiments were performed on the cell bulk, treated or not for 72 h with 1 µg/mL doxycycline for shRNA induction.

Lentiviral transduction was carried out with lentiviral vectors (6 µg), 20 µL of lipofectamine 2000, psPLex1 (4 µg, provides packaging proteins), and mPMD2.G (2 µg, provides VSV-g envelope protein) plasmids into 293 T cells to produce lentiviral particles. Then, 72 h after cell transfection, 2 mL of the supernatants containing the virus particles were collected and added to 2 × 10^6 MOLM14 cells in a six-well plate. Polybrene (6 µg/mL) was added and spinoculation was performed by centrifuging cells for 60 min at 800 × g. Six hours after transduction, the medium containing the viruses was removed. Then, after an additional 72 h, transduced cells were selected with 1 µg/mL puromycin. All shRNA experiments were performed on the cell bulk, treated or not for 72 h with 1 µg/mL doxycycline for shRNA induction.

Measurement of oxygen consumption in AML cultured cells. OCR was measured at 37 °C using an XF24 extracellular flux analyzer (Seahorse Biosciences, Billerica, MA, USA). The day before the assay, non-mitochondrial respiration was subtracted from the calibration buffer medium supplied by Seahorse Biosciences to hydrate over- night. Seahorse XFp microplates wells were coated with 25 µL of Cell-Tak (Corning: 354240) solution at a concentration of 22.4 µg/mL and kept at 4 °C overnight. A total of 2 × 10^6 cells per well were allowed to Seahorse microplates and rested for 1 h in XF base minimal DMEM media containing 1 µM glucose, 1 mM pyruvate, and 2 mM glutamine before rates of glycolysis and oxidative phosphorylation were determined. Initially, baseline cellular OCR is measured, from which basal respiration can be derived by subtracting non-mitochondrial respiration. Next, oligomycin, a complex V inhibitor, is added and the resulting OCR is used to derive ATP-linked respiration (by subtracting non-mitochondrial respiration from the oligomycin rate). Lastly, and rotenone, inhibitors of complex III and I, are added to shut down ETC function, revealing the non-mitochondrial respiration. To determine the part of the OCR dependant on FAO, 3 µM of Etx was added to wells for 45 min of resting and 15 min before the OCR measurement. For oleate- induced OCR, cells were preincubated in Hank’s Balanced Salt Solution (HBSS) supplemented with 20 µM of BSA or 20 µM of oleate-BSA for 1 h; subsequently, a readout of basal OCR was measured.

Transcriptomic analysis. Transcriptome profiling assays on MOLM14 and U937 cells untreated or treated with 10 mM metformin 24 h in independent experiments were performed using the Affymetrix HuGene 2.0_starrays (GSE97346) RNA-sequencing subjected to differential expression analysis with a minimum of an absolute fold change of > 2.0 and a false discovery rate < 0.05, applying Benjamini–Hochberg procedure.

Lipidomic analysis. Total lipid extracts were prepared following Bligh and Dyer procedures, then analyzed by gas chromatography/mass spectrometry for neutral lipid and fatty acid content. The total concentration of triglycerides among all the neutral lipids measured: total triglycerides (C49-TG + C51-TG + C53-TG + C55-TG + C57-TG + C59-TG) + total cholesterol ester (Chlo-C16 + Chlo-C18 + Chlo-C20-4).

Animal Care and Use Committee of Région Midi-Pyrénées (France). Genes were considered significantly downregulated in MOLM14 (221 genes) and U937 (636 genes) were analyzed using Genomatix software (Genome Analyser bioinformatics program, www.genomatix.de, Munich, Germany) for further functional analyses (GO term) based on the Genomatix literature mining. GeneTool software (https://www.syngene.com/support/software- downloads/), Genomatix software (https://www.genomatix.de/), FlowJo (https://www.becton.com/software/flowjo/downloads), and Wave (https://www.agilent.com/en/products/cell-analysis/software- download-for-wave-desktop).
Statistical analysis. Data from at least three independent experiments are reported as means ± standard error of the mean. Unless otherwise indicated, unpaired two-tailed Student’s t-tests or paired t-tests for patients’ studies analysis were carried out with Prism 8 software (GraphPad Software Inc). The Kaplan–Meier method was used to estimate leukemia-free survival in xenografted mice. Log-rank p-values (Mantel–Cox test) were used for comparisons of leukemia-free survival. For GenoAnalyzer bioinformatics tool (Genomatix) the significance of the association between each GO term list was measured by Fisher’s exact test.

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

Data availability
Transcriptome profiling assays on MOLM14 and U937 treated or not with 10 mM metformin in independent triplicates. GEO: GSE97346. Source data are provided with this paper for Figs. 1–6 and Supplementary Figs. 1–8. Raw data are available on reasonable request.

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**Author contributions**

C.B., N.B., C.J., and J.-E.S. conceived and designed the experiments. C.B., N.B., and C.J. carried out most experiments. E.S. and T.F. performed Western blots experiments. A.B. and J.B.-M. evaluated the level of triglycerides. F.B. established the protocol for lipogenesis quantification. C.J., J.-E.S., N.B., C.B., E.S., T.F., S.M., C.R., S.B., and V.M.-D.M. analyzed and interpreted the data. C.J. and J.-E.S. wrote the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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**Correspondence** and requests for materials should be addressed to J.-E.S. or C.J.

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