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Autophagy-deficient mice are more susceptible to engrafted leukemogenesis

Chaorong Ge[1], Ni An[1], Lei Li[1], Wen Wei, Li Ji, Na Yuan, Yixuan Fang, Li Xu, Lin Song, Jingyi Zhang, Chenglin Song, Jianrong Wang*, Suping Zhang*

Hematology Center of Cyrus Tang Medical Institute, Jiangsu Institute of Hematology, Institute of Blood and Marrow Transplantation, Collaborative Innovation Center of Hematology, Key Laboratory of Stem Cells and Biomedical Materials of Jiangsu Province and Chinese Ministry of Science and Technology, State Key Laboratory of Radiation Medicine and Radioprotection, Soochow University, Suzhou 215123, China

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ABSTRACT

Autophagy is primarily considered as an important survival mechanism for both normal cells and cancer cells in response to metabolic stress or chemotherapy; but the role of autophagy in leukemogenesis is not fully understood. The aim of this study is to explore the role of intrinsic autophagy in the leukemogenesis of B-cell acute lymphoblastic leukemia (B-ALL). In this study, conditional knockout mice Atg7$^{f/f}$;Ubc-Cre, in which an autophagy-essential gene Atg7 is universally deleted, were used as recipients, B-ALL cell line 697 was used as donor cells to generate leukemia mouse model. Compared to wild-type mice, Atg7 knockout mice were more susceptible to engrafted leukemogenesis, shown by increase in white blood cells, lymphocytes, and platelets, decrease in HSPC number and its colony-forming unit (CFU). The liver and spleen displayed hepatosplenomegaly and inflammatory cell infiltration. Furthermore, second competitive transplantation revealed dysfunction of the HSPC in Atg7-knockout leukemia mice represented by destructive self-renew ability (CFU) and reconstitution ability including decreased B220, Ter 119 cells, and increased Gr-1 cell percentage. In summary, Mice with universal deletion of Atg7 are more inclined to the occurrence of engrafted human leukemia, which is largely attributed to the deterioration of the function of HSPC in autophagy deficient mice.

1. Introduction

Autophagy is a well-defined cellular process involved in many physiological and pathological processes. It is induced under many different conditions such as cellular stress and organelle turnover, being responsible for the maintenance of the cellular homeostasis, caloric balance and development. However, several other functions of autophagy have been demonstrated in numerous pathological processes such as infectious diseases, cardiomyopathy, neurodegenerative diseases, diabetes, diseases associated to aging and cancer. Regarding the latter, autophagy has a dual function, acting as a cell survival mechanism (favoring the growth of established tumors) and as a tumor suppressor (preventing the accumulation of damaged proteins and organelles) [1]. Autophagy is known to be implicated in cancer as both a tumor promoter and a tumor suppressor [2]. Moreover, several studies have shown that autophagy plays an important role as a cellular mechanism mediating sensitization to cancer therapy in cancer cell lines, being a useful strategy for the treatment of drug resistant tumors [3–7].

In hematologic malignancies, autophagy either acts as a chemoresistance mechanism or has tumor suppressive functions, depending on the context [8]. B-ALL accounts for the most cancer incidences in children. We've recently found that autophagy is down-regulated in childhood B-ALL [9], suggesting a possible link between autophagy failure and pediatric B-ALL leukemogenesis. We also found that activation of autophagy by rapamycin inhibits *in vitro* pre-B ALL cells in part through down-regulating DNA and RNA polymerases [10]. Using a B-ALL xenograft mouse model bearing human B-ALL 697 cells, we found that pharmacologically induced autophagy collaborates with ubiquitination in the degradation of oncoprotein E2A/Pbx1, thereby inhibiting the B-ALL cells [9]. It has become increasingly important to specifically determine whether intrinsic autophagy has an important role in counteracting B-ALL. Therefore, we sought to dissect the role of intrinsic autophagy through the *in vivo* homozygous deletion of Atg7 in human B-ALL 697 cell-driven murine leukemia model.

* Corresponding authors at: Cyrus Tang Hematology Center, 199 Ren'ai Road, Industrial park, Suzhou 215123, China.
E-mail addresses: jrwang@suda.edu.cn (J. Wang), spzhang@suda.edu.cn (S. Zhang).
[1] These authors contributed equally to this work.

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2. Materials and methods

2.1. Cell lines and mice

Acute lymphoblastic leukemia cell line 697 was used for generation of leukemia mouse model. The cells were grown in RPMI1640 medium (Hyclone, South Logan, UT) with 10% fetal bovine serum (Gibco, Grand Island, NY) in 37 °C, 5% CO_2 incubator.

$Atg7^{f/f}$;Ubc-Cre and CD45.1 mice were used in this study. All mice were bred and housed in the specific pathogen free (SPF) animal facilities of Soochow University. $Atg7^{f/f}$ mice kindly provided by Dr. Komatsu [11] were crossed to Ubc-Cre mice (Jackson Laboratory, Sacramento, CA) to obtain $Atg7^{f/f}$;Ubc-Cre mice. Genotyping was performed on tail genomic DNA. Male and female mice were used equally in all experiments, and littermates were always used as controls. CD45.1 mice were from Jackson Laboratory. All experiments with animals were approved by Soochow University Institutional Animal Care and Use Committee.

2.2. Genotyping and western blot

$Atg7^{f/f}$;Ubc-Cre genotyping was performed on tailgenomic DNA. Atg7 gene of the mice was deleted by injecting Tamoxifen (Sigma-Aldrich, St. Louis, MO) i.p. at a dose of 0.5 mg per day for one week when the mice were three weeks old. After induction by tamoxifen for one week or four weeks, different tissues of wild type ($Atg7^{+/+}$), heterozygote ($Atg7^{+/-}$)and homozygote ($Atg7^{-/-}$)mice including blood, bone marrow, heart, lung, kidney, liver and spleen were collected, the DNA and protein were extracted. Genotyping and protein expression (ATG7 and LC3) of different tissues were detected by PCR and western blot.

For PCR amplification of Atg7 and Ubc-Cre, the primers and band sizes are listed in Table 1.

For western blot, cellular proteins were extracted by lysing cells in extraction buffer (RIPA lysate from CST plus protease inhibitor from Roche). The protein concentration was determined by BCA assay (Perice, thermo fisher, Rockford, IL). Equal amounts of protein (50 μg) were fractionated by electrophoresis in SDS-polyacrylamide gel. The proteins were subsequently transferred to PVDF membranes. Antibodies against ATG7 (Cell Signaling Technology, Danvers, MA), LC3 (Novus Biologicals, Littleton, CO) and GAPDH (Cell Signaling Technology) were applied to probe the membranes, respectively. The secondary antibodies (anti-rabbit or anti-mouse) (Cell Signaling Technology) conjugated to horseradish peroxidase were used. Signals were detected using the ECL system (Biological Industry, Kibbutz Beit Haemek, Israel).

2.3. Generation of mouse leukemia model

Wild-type (WT, $Atg7^{+/+}$) and Atg7 knockout (KO, $Atg7^{-/-}$) mice were used in the leukemia model establishment experiment. There were at least 6 mice per group with male and female equally unless indicated in the experiments. To receive 697 human leukemia cells, mice were exposed to 3 Gy γ ray radiation to destroy the immune system. 5×10^6 697 cells were injected by i.v. to each mouse for leukemia genesis. Control mice received PBS instead of 697 cells. 10 to 12 weeks after 697 cell injection, the mice were sacrificed for detection of leukemia and hematopoiesis. Peripheral blood was obtained from tail vein of mice, 20 μl blood was diluted and counted with blood cell counter Sysmex KX21N (Sysmex, Kobe, Japan) for white blood cell (WBC), lymphocyte (LYM), red blood cell (RBC), hemoglobin (HGB) and platelet (PLT) analysis. The mice module was selected to measure the mice blood cells.

Liver and spleen were obtained. The weights were recorded and liver/spleen coefficients were calculated as weights of liver/spleen to weights of body. Parts of them (3 cm^3 for liver and 1 cm^3 for spleen) were fixed in 4% paraformaldehyde for pathological observation with Hematoxylin and eosin (HE) stain, and part of liver (100 mg) was lysed for protein extraction. RIPA lysate from CST plus protease inhibitor (Roche, Basel, Switzerland) were used for protein extraction. ATG7 and LC3 of liver protein were measured by western blot.

Table 1
Primers used in this study.

Primer target	Primer name	Primer sequence	Band size
Atg7	Atg7-F	CATCTTGTAGCACCTGCTGACCTGG	WT: 653 bp
	Atg7-R	CCACTGGCCCATCAGTGAGCATG	Flox-p:
	Floxp-R	GCGGATCCTCGTATAATGTATGC TATACGAAGTTAT	426 bp
Ubc-Cre	Ubc-Cre-F	GCGGTCTGGCAGTAAAAACTATC	900 bp
	Ubc-Cre-R	GTGAAACAGCATTGCTGTCACTT	
KO	KO-F	TGGCTGCTACTTCTGCAATGATGT	Flox-p:
	KO-R	AAGCCAAAGGAAACCAAGGGAGTG	2000 bp
			WT: 1641 bp
			KO: 600 bp

2.4. Detection of hematopoietic cell number and function

After mice sacrifice, bone marrow (BM) cells were flushed by a 25-gauge needle from the long bones (tibias and femurs) with HBSS without calcium or magnesium (Invitrogen, Life Technology, Grand Island, NY, USA). BM monocytes were isolated with Ficoll density gradient centrifugation (BM suspension was added to Ficoll solution at 1:1 ratio and centrifuged at 400*g* for 30 min, the middle white layer was extracted as BM monocytes). For HSC analysis, lineage negative cells were enriched with magnetic-activated cell sorting (MACS) lineage kit (MiltenyiBiotec, Bergisch Gladbach, Germany) according to the manufacture's protocol, and stained with Sca-1 PE, c-Kit APC. LSK ($Lin^-Sca\text{-}1^+c\text{-}Kit^+$) cells were defined as hematopoietic stem and progenitor cells. All the antibodies were obtained from BD (BD Pharmingen, San Diego, CA); and analyzed by flow cytometry (BD Calibur or BD FACS Aria III). HSC function of clonogenic progenitors was determined with colony forming unit assay (CFU) in methylcellulose medium (Stem Cell Technologies, Vancouver, BC, Canada) using 1×10^4 bone marrow mononuclear cells per dish (35 mm) and incubated in a humidified atmosphere with 5% CO_2 at 37 °C for 7 days. The number of colonies containing more than 50 cells was determined.

2.5. Second competitive transplantation

To further determine the function of HSC in the leukemia mouse model, second competitive transplantation was explored. BM cells from different leukemia groups (CD45.2 mice) plus normal CD45.1 mice were used as donors. Lethally irradiated CD45.1 mice were used as recipients. The CD45.2 donor mice were divided into four groups of WT, WT + 697, Atg7 KO control and Atg7 KO + 697 with 3 to 4 mice per group and 10 to 12 weeks after 697 leukemia cell injection, the mice were sacrificed and bone marrow cells were extracted and pooled in each group, bone marrow cells of normal CD45.1 mice were obtained simultaneously. 1×10^6 CD45.2 cells and 1×10^6 CD45.1 cells were mixed and injected into 8.5 Gy irradiated CD45.1 mice by i.v. with 5 mice per group. Four weeks after the transplantation, the CD45.1 recipient mice were sacrificed and the function of HSC was analyzed, including the percentage of CD45.2 and CD45.1 in BM cells, percentage of LSK cells, CFU ability of BM cells, and HSC differentiation ability detected by percentage of B220, Gr-1, Ter119 cells by flow cytometry.

3. Results

3.1. Universal deletion of Atg7 gene in mice

Ubc-Cre directed universal deletion of Atg7 gene was attempted. Mice of $Atg^{f/f}$, $Atg^{f/wt}$;Ubc-Cre and $Atg7^{f/f}$;Ubc-Cre were injected for

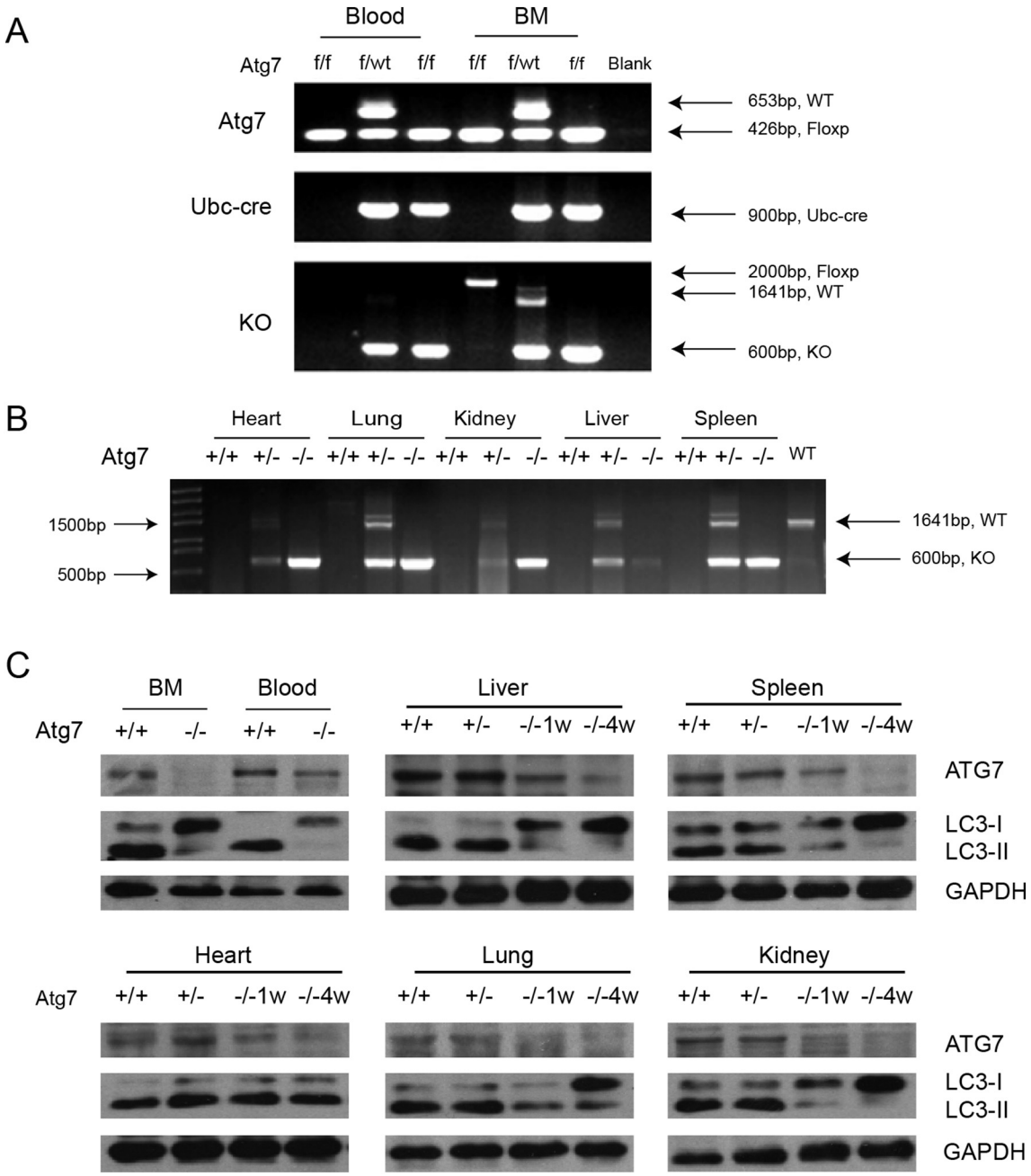

Fig. 1. Universal deletion of Atg7 gene in mice. Mice of $Atg7^{f/f}$, $Atg7^{f/wt}$;Ubc-Cre and $Atg7^{f/f}$;Ubc-Cre were injected for tamoxifen everyday for one week to induce the knockout of Atg7 gene. (A) PCR results of blood and bone marrow. (B) PCR results of heart, lung, kidney, liver and spleen. Only one KO band was showed in all of the tissues of the $Atg7^{f/f}$;Ubc-Cre mice, indicating that Atg7 gene was deleted in the mice. (C) Western blot of ATG7 and LC3 protein in different tissues, ATG7 protein was down-regulated in most tissues, especially in 4 weeks after induction. LC3-II was decreased in blood, bone marrow, liver, spleen, kidney, and lung, but not obviously in heart.

Tamoxifen daily for one week to induce the knockout (KO) of Atg7 gene. One week after induction, DNA of different tissues including blood, bone marrow, heart, lung, kidney, liver and spleen were extracted for DNA genotyping. For PCR detection of Atg7 and Ubc-Cre, as shown in Fig. 1A and B, there is only one KO band in all the tissues of the $Atg7^{f/f}$;Ubc-Cre mice, indicating that Atg7 gene was deleted in the mice.

As for ATG7 and LC3 protein expression, protein of bone marrow and blood cells were extracted at four weeks after tamoxifen injection, proteins of liver/spleen/heart/lung/kidney were extracted at one week and four weeks after tamoxifen injection. Western blot results (Fig. 1C) showed that ATG7 protein was down-regulated in most tissues, especially in four weeks after induction. LC3-II was barely accumulated in blood, bone marrow, liver, spleen, kidney, and lung, but not obviously in heart, probably due to its tolerance. These results indicate that injection of tamoxifen efficiently induced autophagy defect in most tissues, in particular in hematopoietic system of $Atg7^{f/f}$;Ubc-Cre mice.

3.2. Mice with Atg7 deletion acquired an increased risk of engrafted leukemogenesis

Atg7 knockout mice (Atg7 KO, $Atg7^{-/-}$) were used as experimental group, Wild-type mice (WT, $Atg7^{+/+}$) were used as control group. B-ALL 697 cells were injected by i.v. to generate mouse leukemia model.

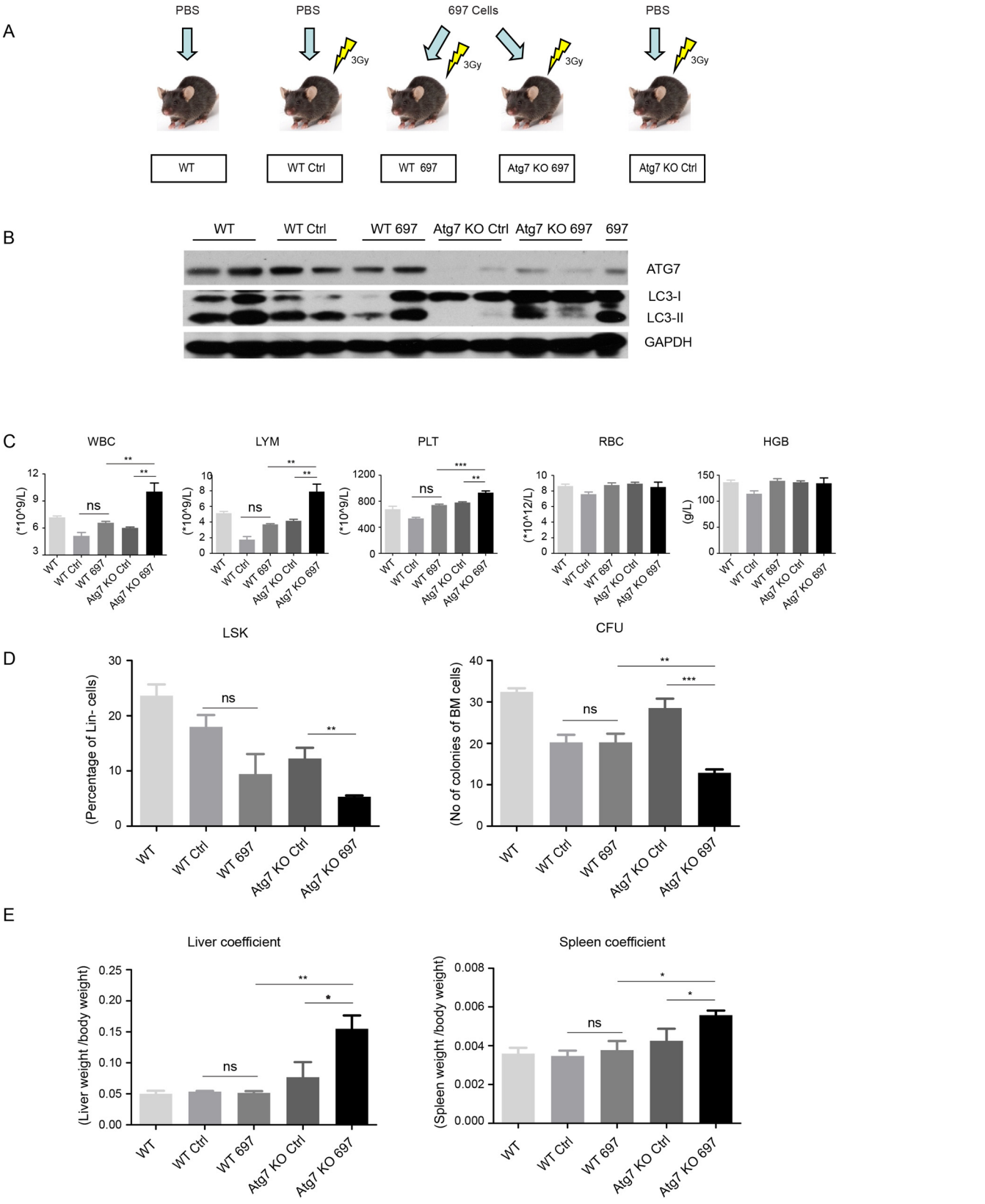

Fig. 2. Mice with Atg7 deletion acquired an increased risk of the occurrence of engrafted human leukemia. (A) The method of leukemia model establishment. (B) ATG7 and LC3 protein expression in different groups, as can be seen that ATG7 was depleted in Atg7 KO mice, as well as LC3-II. The 697 leukemia cells were used as control. (C) Peripheral blood cell count in different groups. WBC, LYM, PLT were increased significantly in the Atg7 KO 697 leukemia group compared to Atg7 KO control group and WT 697 leukemia group. (D) HSC and CFU assay. HSC-enriched LSK cell number decreased significantly in the Atg7 KO 697 leukemia group compared to Atg7 KO control group. CFU decreased significantly in the Atg7 KO 697 leukemia group compared to Atg7 KO control group and WT 697 leukemia group. (E) Liver and spleen coefficient in different groups. Liver and spleen coefficient increased significantly in the Atg7 KO 697 leukemia group compared to Atg7 KO control group and WT 697 leukemia group.
*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

PBS was injected as their individual inner control group. Fig. 2A illustrated the procedure for generating leukemia animal model.

10 to 12 weeks after cell injection, whereas ATG7 and LC3 protein expression were shown in different groups, ATG7 protein was depleted in Atg7 KO mice, along with the blocked conversion of LC3-II from LC3-I. The 697 cells were used as positive control for western blot (Fig. 2B). Peripheral blood cell count results showed that the number of WBC, LYM and PLT in Atg7 KO leukemia mouse model group increased significantly compared to Atg7 KO control and WT 697 groups (Fig. 2C). There was no difference between WT 697 group and WT control group.

As compared with Atg7 KO control, the Atg7 KO 697 mice displayed a significant reduction in the percentage of HSC-enriched LSK ($Lin^{-}Sca\text{-}1^{+}c\text{-}Kit^{+}$) population over lineage negative bone marrow cells, whereas no significant difference was found between WT control and WT 697 mice (Fig. 2D, left). Furthermore, *in vitro* repopulation capacity using colony forming unit assay in Atg7 KO 697 mice significantly decreased as compared with Atg7 KO control, but no change was detected between WT control and WT 697 mice (Fig. 2D, right). The data suggest that autophagy defect deteriorates the maintenance of HSC-enriched LSK population and their repopulation capacity in the engrafted leukemia mouse model.

The liver and spleen coefficient showed that liver and spleen in Atg7 KO 697 model group increased significantly compared to other groups (Fig. 2E), indicating hepatosplenomegaly in Atg7 KO leukemia model. The histology section of liver and spleen (Fig. 3) showed that there was no pathological difference between WT control and WT 697 groups, but adipose degeneration and liver cell necrosis aroused in liver 8 weeks after 697 cell injection; the normal structure of liver disappeared in the Atg7 KO 697 leukemia model mice, and it became severer in 12-week mice. The spleen histology showed abnormal structure in 12weeks after 697 cell injection.

The diagnosis of lymphocytic leukemia includes an increase in WBC, LYM, and appearance of naïvelymphocytes in peripheral blood, hepatosplenomegaly and inflammatory cell infiltration, as well as dysfunction of hematopoietic stem cells [12,13]. Our results are mostly accordance with the symptoms of lymphocytic leukemia and indicate that the Atg7 KO mice were more sensitive to acquire leukemia compared to WT mice by injecting leukemia 697 cells.

3.3. Autophagy deficiency deteriorates the function of HSPC in the engrafted human leukemia mouse model

The cell number and function of HSPC, represented by LSK markers and CFU (colony formation unit), decreased significantly in Atg7 KO 697 model group compared to Atg7 KO control and WT 697 groups, indicating the dysfunction of the HSPC in the Atg7 depleted leukemia mice.

To further investigate the HSPC function of the mice in different groups, second competitive transplantation of BM cells was explored. BM cells of WT and Atg7 KO mice (CD45.2 mice) injected w/o leukemia 697 cells were used as donors, BM cells of CD45.1 mice were used as helper cells, and lethally irradiated CD45.1 mice were used as recipients (Fig. 4A). Flow cytometry results showed that CD45.2 cells decreased and CD45.1 cells increased significantly in Atg7 KO 697 leukemia group compared to other groups (Fig. 4B), indicating that the repopulated bone marrow cells in the lethally irradiated recipient mice were largely from the CD45.1 helper mice, and the CD45.2 donor cells from this group were destroyed. The repopulated HSPC function represented by LSK markers and CFU (Fig. 4C) also decreased in Atg7 KO 697 leukemia mouse group compared to other groups. The differentiation ability of HSPC represented by B220 (B cells), Ter119 (Red blood cells) and Gr-1 (Granulocytes) cell percentage in bone marrow cells showed that B220 and Ter119 decreased but Gr-1 increased in Atg7 KO leukemia group compared to WT control and Atg7 KO control, but only B220 cells in WT 697 leukemia group decreased compared to WT control. These results indicate that in Atg7 KO mice, the HSPC number and function was more susceptible to ectogenic leukemia cells compared to WT mice, and leukemia arises more easily from the engrafts in the autophagy-deficient mice.

4. Discussion

Autophagy is a catabolic pathway that involves lysosomal degradation and recycling of proteins and organelles [14], and is therefore considered an important survival mechanism for both normal cells and cancer cells in response to metabolic stress or chemotherapy. As the role of autophagy in cancer, to be tumor promoter or tumor suppressor, is dependent on the context and the stage of tumor [15]. In established tumors, autophagy acts as a cell survival mechanism [16], but in normal cells, autophagy may prevent the accumulation of damaged

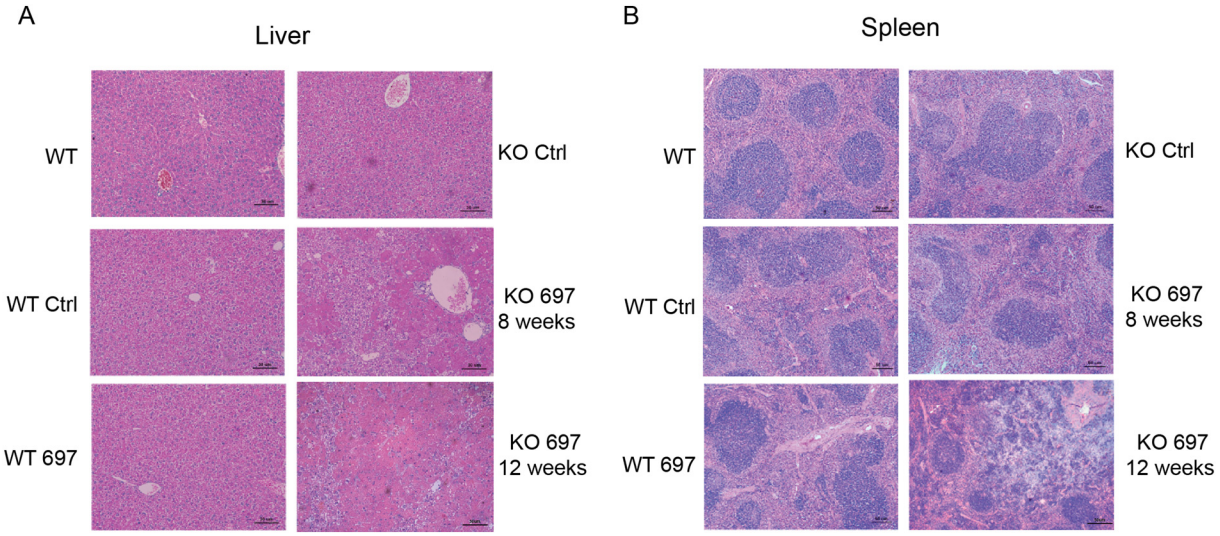

Fig. 3. HE section of liver and spleen in different groups. (A) Liver pathological change in different groups. (B) Spleen pathological change in different groups. There was no pathological difference between WT control and WT 697 leukemia groups, but adipose degeneration and liver cell necrosis increased in liver 8 weeks after the 697 leukemia cell injection, and the normal structure of liver disappeared in the Atg7 KO leukemia mice, and it became severer in 12-week mice. The spleen histology showed abnormal structure in 12 weeks after the 697 leukemia cell injection.

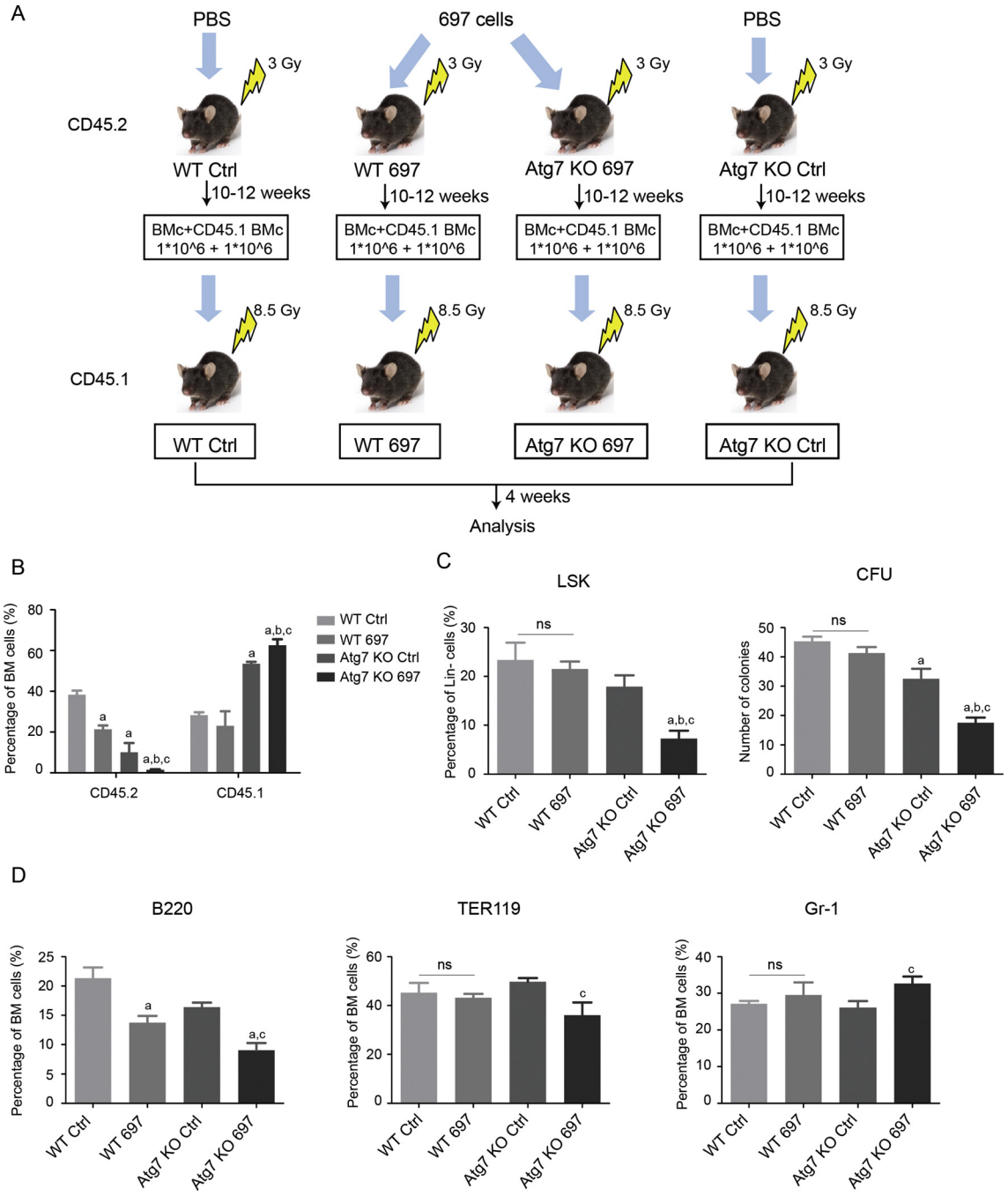

Fig. 4. Autophagy deficiency deteriorates the function of HSPCs in the engrafted human leukemia mouse model. (A) The illustration figure of competitive transplantation. BM cells of WT and Atg7 KO mice (CD45.2 mice) injected w/o leukemia 697 cells were used as donors, BM cells of CD45.1 mice were used as helper cells, and lethally irradiated CD45.1 mice were used as recipients. (B) CD45.2 and CD45.1 cell percentage by flow cytometry, CD45.2 cells decreased and CD45.1 cells increased significantly in Atg7 KO leukemia group compared to other groups, indicating that the repopulated bone marrow cells in the lethally irradiated recipient mice were most from the CD45.1 helper mice, and the CD45.2 donor cells from this group were destroyed. (C) HSPC function represented by LSK number and CFU. HSPC function decreased significantly in Atg7 KO leukemia mouse group compared to other groups. (D) The differentiation ability of HSPC represented by B220 (B cells), TER119 (Red blood cells) and Gr-1 (Granulocytes) cell percentage in bone marrow cells. It showed that B220 and TER119 decreased but Gr-1 increased in Atg7 KO leukemia group compared to WT control and Atg7 KO control, B220 cells in WT 697 leukemia group decreased compared to WT control.
a: compared to WT control, $p < 0.05$; b: compared to the WT 697 group, $p < 0.05$; c: compared to Atg7 KO control, $p < 0.05$.

proteins and organelles, so autophagy acts as a tumor suppressor in normal cells [17]. Autophagy defect may lead to anemia, long-term follow-up myelodysplasia or malignant transformation [18–20]. Recent studies in myeloid leukemia have suggested that in acute myeloid leukemia the autophagy machinery might be disrupted, resulting in intracellular accumulation of damaged mitochondria and increased levels of reactive oxygen species (ROS), with high ROS levels potentially promoting leukemic transformation [21–23]. In contrast, other studies have shown that leukemic cells require functional autophagy during leukemia maintenance [24–28]. In addition, autophagy can be an escape mechanism utilized by leukemic cells after treatment with chemotherapeutics such as mTOR and HDAC inhibitors [29–33].

To study the role of autophagy in specific physical or pathological process, it is necessary to create an autophagy defect animal model. In

this study we generated a conditional knockout mouse of $Atg7^{f/f}$;Ubc-Cre, in which Atg7 gene was universally deleted in most tissues after tamoxifen induction which could be seen from the PCR and western blot results. The 697 leukemia cell transplant results showed that Atg7 KO 697 leukemia mouse group has the most serious symptoms compared to other groups, indicating that the Atg7 deficient mice were more sensitive to acquire leukemia compared to WT mice by injecting leukemia 697 cells. It suggests that deficiency of autophagy may result in malignancy in normal cells and tissues before tumor formation, which is consistent with the suppressive role of autophagy.

HSCs are the source of all kinds of progeny blood cells, whose dysfunction will lead to multiple malignant blood disorders [34]. The genetic ablation of autophagy has been shown to result in severe impairments to HSPC maintenance in mice [35–37]. Autophagy depletion caused decreased number and function of HSPCs in Atg7 deficient mice, which may be responsible for the increased sensitivity of autophagy deficient mice to the engrafted leukemia cells. The competitive transplantation experiment subsequently showed the worst function of HSPCs in autophagy deficient leukemia mouse model group, both in self-renewal and in differentiation of HSPCs. Then how the dysfunction of HSPCs leads to leukemia? Since HSCs exist alongside with several cell types such as endothelial cells, mesenchymal cells and osteoblasts in the BM microenvironment which comprise the stem cell niche, these cells might regulate HSC function and can contribute to leukemogenesis [38]. Endothelial cells modulate HSC function *via* Notch and other signaling pathways such as interleukin-4 to regulate leukemogenesis [39]. Dysfunction of the MSC stromal cells can induce or alter the progression of hematologic malignancies or regulate leukemia stem cells [40]. Other cell types such as osteoblasts, adipocytes, macrophages and megakaryocytes might also affect HSC function and contribute to the malignancy [38]. In the study of Hu et al. [41], it showed that in Notch1-induced mice leukemia model, hematopoietic cells in the leukemic environment are progressively decreased as measured by both phenotype and function. However, the effects of the leukemic environment on HSCs and HPCs are distinct in that the repopulation potential of HSCs from the leukemic environment is preserved, whereas mature blood cells cannot be produced because of the exhaustion of HPCs. In our study, the phenotype and function of HSPCs which includes both HSCs and HPCs, were suppressed in Atg7 KO leukemia mice, which was mostly due to the autophagy deficiencies of the mice, so it's more vulnerable to leukemia cells. But the detailed mechanisms and the different functions of HSCs and HPCs and how they are regulated during leukemogenesis need to be further explored.

In summary, in the present study, by using autophagy gene conditional knockout mouse model, we found that mice with Atg7 deletion are more inclined to the occurrence of engrafted human leukemia, largely due to deterioration of the function of HSPC in autophagy deficient mice, suggesting that maintenance of autophagy capacity in the normal HSPCs of leukemia hosts is important to delay or restrain from the progression of leukemia.

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Author contribution

CG, NA and LL performed the majority of the experiments. WW and LJ assisted with flow cytometry experiments. NY and YF performed the animal breeding and genotyping. LX helped with cell transplantation. LS helped with cell culture and western blot. JZ and CS assisted with some of the experiments. CG, JW and SZ contributed to experimental design, data analysis, and manuscript preparation.

Conflict of interest

The authors declare no potential conflict of interest.

References

[1] N. Ruocco, S. Costantini, M. Costantini, Blue-print autophagy: potential for Cancer treatment, Mar. Drugs 14 (2016), https://doi.org/10.3390/md14070138 pii: E138.
[2] L. Galluzzi, F. Pietrocola, J.M. Bravo-San Pedro, R.K. Amaravadi, E.H. Baehrecke, F. Cecconi, P. Codogno, J. Debnath, D.A. Gewirtz, V. Karantza, A. Kimmelman, S. Kumar, B. Levine, M.C. Maiuri, S.J. Martin, J. Penninger, M. Piacentini, D.C. Rubinsztein, H.U. Simon, A. Simonsen, A.M. Thorburn, G. Velasco, K.M. Ryan, G. Kroemer, Autophagy in Malignant Transformation and Cancer Progression, 34 (2015), pp. 856–880, https://doi.org/10.15252/embj.201490784.
[3] S. Aveic, G.P. Tonini, Resistance to receptor tyrosine kinase inhibitors in solid tumors: can we improve the cancer fighting strategy by blocking autophagy? Cancer Cell Int. 16 (2016) 62, , https://doi.org/10.1186/s12935-016-0341-2.
[4] A. Duffy, J. Le, E. Sausville, A. Emadi, Autophagy modulation: a target for cancer treatment development, Cancer Chemother. Pharmacol. 75 (2015) 439–447, https://doi.org/10.1007/s00280-014-2637-z.
[5] D.A. Gewirtz, The four faces of autophagy: implications for cancer therapy, Cancer Res. 74 (2014) 647–651, https://doi.org/10.1158/0008-5472.CAN-13-2966.
[6] D.A. Gewirtz, Autophagy and senescence in cancer therapy, J. Cell. Physiol. 229 (2014) 6–9, https://doi.org/10.1002/jcp.24420.
[7] R. Langer, C.J. Streutker, P.E. Swanson, Autophagy and its current relevance to the diagnosis and clinical management of esophageal diseases, Ann. N. Y. Acad. Sci. 1381 (2016) 113–121, https://doi.org/10.1111/nyas.13190.
[8] A. Nencioni, M. Cea, F. Montecucco, V.D. Longo, F. Patrone, A.M. Carella, T.L. Holyoake, G.V. Helgason, Autophagy in blood cancers: biological role and therapeutic implications, Haematologica 98 (2013) 1335–1343, https://doi.org/10.3324/haematol.2012.079061.
[9] N. Yuan, L. Song, W. Lin, Y. Cao, F. Xu, S. Liu, A. Zhang, Z. Wang, X. Li, Y. Fang, H. Zhang, W. Zhao, S. Hu, J. Wang, S. Zhang, Autophagy collaborates with ubiquitination to downregulate oncoprotein E2A/Pbx1 in B-cell acute lymphoblastic leukemia, Blood Cancer J. 5 (2015) e274, https://doi.org/10.1038/bcj.2014.96.
[10] Z. Wang, F. Xu, N. Yuan, Y. Niu, W. Lin, Y. Cao, J. Cai, L. Song, X. Li, Y. Fang, W. Zhao, S. Hu, S. Chen, S. Zhang, J. Wang, Rapamycin inhibits pre-B acute lymphoblastic leukemia cells by downregulating DNA and RNA polymerases, Leuk. Res. 38 (2014) 940–947, https://doi.org/10.1016/j.leukres.2014.05.009.
[11] M. Komatsu, S. Waguri, T. Ueno, J. Iwata, S. Murata, I. Tanida, J. Ezaki, N. Mizushima, Y. Ohsumi, Y. Uchiyama, E. Kominami, K. Tanaka, T. Chiba, Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice, J. Cell Biol. 169 (2005) 425–434 (Epub 2005 May 2).
[12] L. Pang, Y. Liang, J. Pan, J.R. Wang, Y.H. Chai, W.L. Zhao, Clinical features and prognostic significance of TCF3-PBX1 fusion gene in Chinese children with acute lymphoblastic leukemia by using a modified ALL-BFM-95 protocol, Pediatr. Hematol. Oncol. 32 (2015) 173–181, https://doi.org/10.3109/08880018.2014.983625.
[13] A. Castor, L. Nilsson, I. Astrand-Grundström, M. Buitenhuis, C. Ramirez, K. Anderson, B. Strömbeck, S. Garwicz, A.N. Békássy, K. Schmiegelow, B. Lausen, P. Hokland, S. Lehmann, G. Juliusson, B. Johansson, S.E. Jacobsen, Distinct patterns of hematopoietic stem cell involvement in acute lymphoblastic leukemia, Nat. Med. 11 (2005) 630–637 (Epub 2005 May 22).
[14] B. Levine, D.J. Klionsky, Development by self-digestion: molecular mechanisms and biological functions of autophagy, Dev. Cell 6 (2004) 463–477.
[15] J.K. Altman, A. Szilard, D.J. Goussetis, A. Sassano, M. Colamonici, E. Gounaris, O. Frankfurt, F.J. Giles, E.A. Eklund, E.M. Beauchamp, L.C. Platanias, Autophagy is a survival mechanism of acute myelogenous leukemia precursors during dual mTORC2/mTORC1 targeting, Clin. Cancer Res. 20 (2014) 2400–2409, https://doi.org/10.1158/1078-0432.CCR-13-3218.
[16] R. Grosso, C.M. Fader, M.I. Colombo, Autophagy: a necessary event during erythropoiesis, Blood Rev. 31 (2017) 300–305, https://doi.org/10.1016/j.blre.2017.04.001.
[17] M. Mortensen, E.J. Soilleux, G. Djordjevic, R. Tripp, M. Lutteropp, E. Sadighi-Akha, A.J. Stranks, J. Glanville, S. Knight, S.E. Jacobsen, K.R. Kranc, A.K. Simon, The autophagy protein Atg7 is essential for hematopoietic stem cell maintenance, J. Exp. Med. 208 (2011) 455–467, https://doi.org/10.1084/jem.20101145.
[18] M. Mortensen, D.J. Ferguson, M. Edelmann, B. Kessler, K.J. Morten, M. Komatsu, A.K. Simon, Loss of autophagy in erythroid cells leads to defective removal of mitochondria and severe anemia in vivo, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 832–837, https://doi.org/10.1073/pnas.0913170107.
[19] Y. Cao, S. Zhang, N. Yuan, J. Wang, X. Li, F. Xu, W. Lin, L. Song, Y. Fang, Z. Wang, Z. Wang, H. Zhang, Y. Zhang, W. Zhao, S. Hu, X. Zhang, J. Wang, Hierarchal autophagic divergence of hematopoietic system, J. Biol. Chem. 290 (2015) 23050–23063, https://doi.org/10.1074/jbc.M115.650028.
[20] Y. Cao, J. Cai, S. Zhang, N. Yuan, X. Li, Y. Fang, L. Song, M. Shang, S. Liu, W. Zhao, S. Hu, J. Wang, Loss of autophagy leads to failure in megakaryopoiesis, megakaryocyte differentiation, and thrombopoiesis in mice, Exp. Hematol. 43 (2015) 488–494, https://doi.org/10.1016/j.exphem.2015.01.001.
[21] A.S. Watson, T. Riffelmacher, A. Stranks, O. Williams, J. De Boer, K. Cain,

M. MacFarlane, J. McGouran, B. Kessler, S. Khandwala, O. Chowdhury, D. Puleston, K. Phadwal, M. Mortensen, D. Ferguson, E. Soilleux, P. Woll, S.E. Jacobsen, A.K. Simon, Autophagy limits proliferation and glycolytic metabolism in acute myeloid leukemia, Cell Death Dis. 1 (2015) (pii: 15008).

[22] C. Luo, Y. Li, H. Wang, Z. Feng, Y. Li, J. Long, J. Liu, Mitochondrial accumulation under oxidative stress is due to defects in autophagy, J. Cell. Biochem. 114 (2013) 212–219, https://doi.org/10.1002/jcb.24356.

[23] Y. Sumitomo, J. Koya, K. Nakazaki, K. Kataoka, T. Tsuruta-Kishino, K. Morita, T. Sato, M. Kurokawa, Cytoprotective autophagy maintains leukemia-initiating cells in murine myeloid leukemia, Blood 128 (2016) 1614–1624, https://doi.org/10.1182/blood-2015-12-684696.

[24] A.R. Sehgal, H. Konig, D.E. Johnson, D. Tang, R.K. Amaravadi, M. Boyiadzis, M.T. Lotze, You eat what you are: autophagy inhibition as a therapeutic strategy in leukemia, Leukemia 29 (2015) 517–525, https://doi.org/10.1038/leu.2014.349.

[25] S. Piya, S.M. Kornblau, V.R. Ruvolo, H. Mu, P.P. Ruvolo, T. McQueen, R.E. Davis, N. Hail Jr., H. Kantarjian, M. Andreeff, G. Borthakur, Atg7 suppression enhances chemotherapeutic agent sensitivity and overcomes stroma-mediated chemoresistance in acute myeloid leukemia, Blood 128 (2016) 1260–1269, https://doi.org/10.1182/blood-2016-01-692244.

[26] S. Piya, M. Andreeff, G. Borthakur, Targeting autophagy to overcome chemoresistance in acute myleogenous leukemia, Autophagy 13 (2017) 214–215, https://doi.org/10.1080/15548627.2016.1245263.

[27] L.L. Liu, Z.J. Long, L.X. Wang, F.M. Zheng, Z.G. Fang, M. Yan, D.F. Xu, J.J. Chen, S.W. Wang, D.J. Lin, Q. Liu, Inhibition of mTOR pathway sensitizes acute myeloid leukemia cells to aurora inhibitors by suppression of glycolytic metabolism, Mol. Cancer Res. 11 (2013) 1326–1336, https://doi.org/10.1158/1541-7786.MCR-13-0172.

[28] A.M. Martelli, C. Evangelisti, F. Chiarini, J.A. McCubrey, The phosphatidylinositol 3-kinase/Akt/mTOR signaling network as a therapeutic target in acute myelogenous leukemia patients, Oncotarget 1 (2010) 89–103.

[29] M.L. Torgersen, N. Engedal, S.O. Bøe, P. Hokland, A. Simonsen, Targeting autophagy potentiates the apoptotic effect of histone deacetylase inhibitors in t(8;21) AML cells, Blood 122 (2013) 2467–2476, https://doi.org/10.1182/blood-2013-05-500629.

[30] D. Mahalingam, M. Mita, J. Sarantopoulos, L. Wood, R.K. Amaravadi, L.E. Davis, A.C. Mita, T.J. Curiel, C.M. Espitia, S.T. Nawrocki, F.J. Giles, J.S. Carew, Combined autophagy and HDAC inhibition: a phase I safety, tolerability, pharmacokinetic, and pharmacodynamic analysis of hydroxychloroquine in combination with the HDAC inhibitor vorinostat in patients with advanced solid tumors, Autophagy 10 (2014) 1403–1414, https://doi.org/10.4161/auto.29231.

[31] L. Liu, M. Yang, R. Kang, Z. Wang, Y. Zhao, Y. Yu, M. Xie, X. Yin, K.M. Livesey, M.T. Lotze, D. Tang, L. Cao, HMGB1-induced autophagy promotes chemotherapy resistance in leukemia cells, Leukemia 25 (2011) 23–31, https://doi.org/10.1038/leu.2010.225.

[32] M.V. Stankov, M. El Khatib, B. Kumar Thakur, K. Heitmann, D. Panayotova-Dimitrova, J. Schoening, J.P. Bourquin, N. Schweitzer, M. Leverkus, K. Welte, D. Reinhardt, Z. Li, S.H. Orkin, G.M. Behrens, J.H. Klusmann, Histone deacetylase inhibitors induce apoptosis in myeloid leukemia by suppressing autophagy, Leukemia 28 (2014) 577–588, https://doi.org/10.1038/leu.2013.264.

[33] H. Folkerts, S. Hilgendorf, A.T.J. Wierenga, J. Jaques, A.B. Mulder, P.J. Coffer, J.J. Schuringa, E. Vellenga, Inhibition of autophagy as a treatment strategy for p53 wild-type acute myeloid leukemia, Cell Death Dis. 8 (2017) e2927, , https://doi.org/10.1038/cddis.2017.317.

[34] N. Carlesso, A.A. Cardoso, Stem cell regulatory niches and their role in normal and malignant hematopoiesis, Curr. Opin. Hematol. 17 (2010) 281–286, https://doi.org/10.1097/MOH.0b013e32833a25d8.

[35] Y. Cao, J. Cai, S. Zhang, N. Yuan, Y. Fang, Z. Wang, X. Li, D. Cao, F. Xu, W. Lin, L. Song, Z. Wang, J. Wang, X. Xu, Y. Zhang, W. Zhao, S. Hu, X. Zhang, J. Wang, Autophagy sustains hematopoiesis through targeting notch, Stem Cells Dev. 24 (2015) 2660–2673, https://doi.org/10.1089/scd.2015.0176.

[36] F. Liu, J.Y. Lee, H. Wei, O. Tanabe, J.D. Engel, S.J. Morrison, J.L. Guan, FIP200 is required for the cell-autonomous maintenance of fetal hematopoietic stem cells, Blood 116 (2010) 4806–4814, https://doi.org/10.1182/blood-2010-06-288589.

[37] C.B. Lebovitz, A.G. Robertson, R. Goya, S.J. Jones, R.D. Morin, M.A. Marra, S.M. Gorski, Cross-cancer profiling of molecular alterations within the human autophagy interaction network, Autophagy 11 (2015) 1668–1687, https://doi.org/10.1080/15548627.2015.1067362.

[38] M. Seshadri, C.K. Qu, Microenvironmental regulation of hematopoietic stem cells and its implications in leukemogenesis, Curr. Opin. Hematol. 23 (2016) 339–345, https://doi.org/10.1097/MOH.0000000000000251.

[39] A. Gao, Y. Gong, C. Zhu, W. Yang, Q. Li, M. Zhao, S. Ma, J. Li, S. Hao, H. Cheng, T. Cheng, Bone marrow endothelial cell-derived interleukin-4 contributes to thrombocytopenia in acute myeloid leukemia, Haematologica (2019), https://doi.org/10.3324/haematol.2018.214593 Feb 21. (Epub ahead of print).

[40] P. Agarwal, S. Isringhausen, H. Li, A.J. Paterson, J. He, Á. Gomariz, T. Nagasawa, C. Nombela-Arrieta, R. Bhatia, Mesenchymal niche-specific expression of Cxcl12 controls quiescence of treatment-resistant leukemia stem cells, Cell Stem Cell (2019), https://doi.org/10.1016/j.stem.2019.02.018 Mar 14. (Epub ahead of print).

[41] X. Hu, H. Shen, C. Tian, H. Yu, G. Zheng, R. XuFeng, Z. Ju, J. Xu, J. Wang, T. Cheng, Kinetics of normal hematopoietic stem and progenitor cells in a Notch1-induced leukemia model, Blood 114 (2009) 3783–3792, https://doi.org/10.1182/blood-2009-06-227843.