

The Question of Survival or Death: What Is the Role of Autophagy in Acute Myeloid Leukemia (AML)?

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ABSTRACT

Autophagy plays a critical role in balancing sources of energy in response to harsh conditions and nutrient deprivation. Autophagy allows cells to survive in harsh condition and also serve as a death mechanism. Any dysregulation in autophagy signaling may lead to several disorders. Autophagy has been proposed to explain chemotherapy resistance in acute myeloid leukemia (AML). This signaling pathway can either act as a tumor suppressive function or chemo-resistance mechanism. Conventional chemotherapy drugs enhance apoptosis and indicate clinical benefit, but in some cases, relapse and chemotherapy resistance are observed. In leukemia, autophagy may promote cell survival in response to chemotherapy drugs. Therefore, new strategies by inhibiting or activating autophagy may find a broad application for treating leukemia and may significantly enhance clinical outcomes. In this review, we discussed the dimensional role of autophagy in leukemia.

Keywords: Autophagy; Acute myeloid leukemia (AML); Cell survival; Chemoresistance

INTRODUCTION

The word 'autophagy' means "self-eating". Autophagy consists of three forms, including microautophagy (act as a non-selective lysosomal degradative process)¹, chaperone-mediated autophagy (selectively break down intracellular proteins in lysosomes)^{2,3}, and macroautophagy (the most common form that cellular contents are degraded by lysosomes). Macroautophagy (herein referred to as autophagy) is a process that cells from

double-membrane structures, called autophagosomes, surround cellular contents and then fuse with lysosomes. Various stress conditions such as growth factor and nutrient deprivation^{4,5}, endoplasmic reticulum stress, hypoxia, drugs and radiation are able to activate autophagy⁶. This process maintains cellular homeostasis by removing misfolded, damaged and ubiquitylated proteins^{7,8}. Autophagy genes include Atg 1, 3, 4, 5, 7, 8, 9, 10, 12,

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16, ULK and beclin1. The ubiquitin-like proteins, including Atg8/LC3 and Atg12 have some structural similarity, but do not have obvious homology with ubiquitin. Both proteins are induced by Atg7 and subsequently conjugated to phosphatidylethanolamine or Atg5, respectively. Beclin 1 is one member of a complex that involves the class III phosphatidylinositol (PtdIns)-3-kinase (also known as Vps34) that is stimulatory for the autophagy pathway. Class I PtdIns-3-kinases regulate the induction of the kinases mammalian target of rapamycin (mTOR) and Akt, which are known as an inhibitory for autophagy. Autophagy involved in different human diseases, including infectious diseases, neurodegenerative disorders, and cancers. Autophagy has a role in both cell survival and programmed cell death^{8, 9}. The activity of autophagy in cancer treatment is a double-edged sword. On the one hand, this pathway can be the driving force for the preservation of cancer cells. Meanwhile, this signaling pathway acting as an effective tumor suppressive, promotes efficacious anti-cancer immunity and also keep normal cells from the toxicity of cancer treatments⁵. The complex function of autophagy in cancer continues to appear, and it is important for a scientist to clarify the mechanisms that how autophagy influences tumorigenesis and also therapy response¹⁰.

Acute myeloid leukemia (AML) is defined by an accumulation of abnormal hematopoietic progenitor cells that exhibit the morphology of a certain phase of myeloid differentiation in the peripheral blood and bone marrow¹¹⁻¹³. Autophagy machinery has been proposed to play a critical role in hematopoietic malignancies such as AML¹⁴⁻¹⁶. The latest and conventional regimen (7+3 regimen) used for the treatment of AML consists of daunorubicin, idarubicin, and cytarabine¹⁷. These chemotherapeutic agents have been found to induce autophagy in AML. Therefore, it is important to know the function of autophagy which induced through these agents. Nowadays, scientists use the autophagy activator or autophagy inhibitors in combination with conventional medicine to promote the new strategy for cancer therapy. However, the function of autophagy during leukemia treatment is unknown¹⁸.

The autophagic Machinery

Autophagy is an early degradative pathway that happens in all eukaryotic cells. The molecular machinery of autophagy relies on the recognition of ATG genes. The autophagy process can be divided into four stages: induction autophagy, the formation of autophagosome, degradation of the content within autophagosome, and finally, the release of macromolecules from autophagolysosomes.

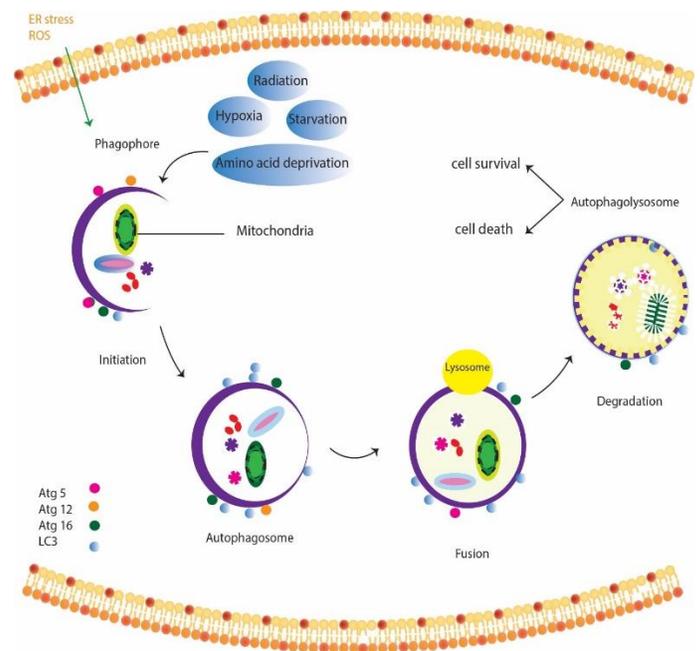


Figure 1. Simple pathway of autophagy

Initiation

Initiation of autophagy involves the ULK protein complex, including Atg13, ULK1,2, and FIP200¹⁹ on the isolation membranes. This complex interacts with other ATG proteins to begin autophagosome formation. The kinase activity of ULK1 is controlled by the negative regulator mTOR complex 1 (mTORC1) during cellular stress²⁰.

Nucleation

Autophagosome formation proceeds require the organization of a large protein complex, known as Class III phosphatidylinositol-3-kinase (PI3K) complex

that interplays with Beclin1, UV irradiation resistance-associated tumor suppressor gene (UVRAG), Atg14, p150, Bcl-2 ambra1, endophilinB1, and PI3K Vacuolar protein sorting 34 (Vps34) that activates PI3K to form phosphatidylinositol 3-phosphate^{7, 8}. Binding beclin 1 to Bcl-2 leads to inhibition of PI3K activity and beclin 1/PI3K complex formation (Beclin 1 in autophagy acts as part of a complex with hVps34/Class III PI3K).

PI3P generation by the Beclin 1/hVps34 complex is critical in the localization of other autophagy proteins to preautophagosomal membranes²¹, but they were activated when UVRAG was added to the complex⁷. Ambra1 through binding to beclin 1 can regulate beclin 1/PI3K complex formation.

Elongation

Elongation of membranes requires Atg3, Atg4, Atg7, Atg10, and an Atg5–Atg12–Atg16L1 complex to conjugate phosphatidylethanolamine to the microtubule-associated protein1 light chain3 (LC3), leading to the translocation of LC3 from the

cytoplasm to the membrane of the forming autophagosomes. In addition, acetylation and Phosphorylation of autophagy machinery proteins provide additional control over autophagosome formation²². After the improvement of autophagosome formation, the Atg proteins are recycled in the cytosol except for LC3 which is bound to the luminal membrane.

Maturation

LC3 associated with the luminal membrane remains in the autophagosome and is degraded to the autolysosome upon maturation, whereas LC3-II can be recycled to the cytoplasmic surface.

Maturation of the autolysosome is characterized by the fusion of autophagosomes with late endosomes, which include lysosomal organelles, multivesicular endosomal bodies, and dissolution of the inner membrane²³.

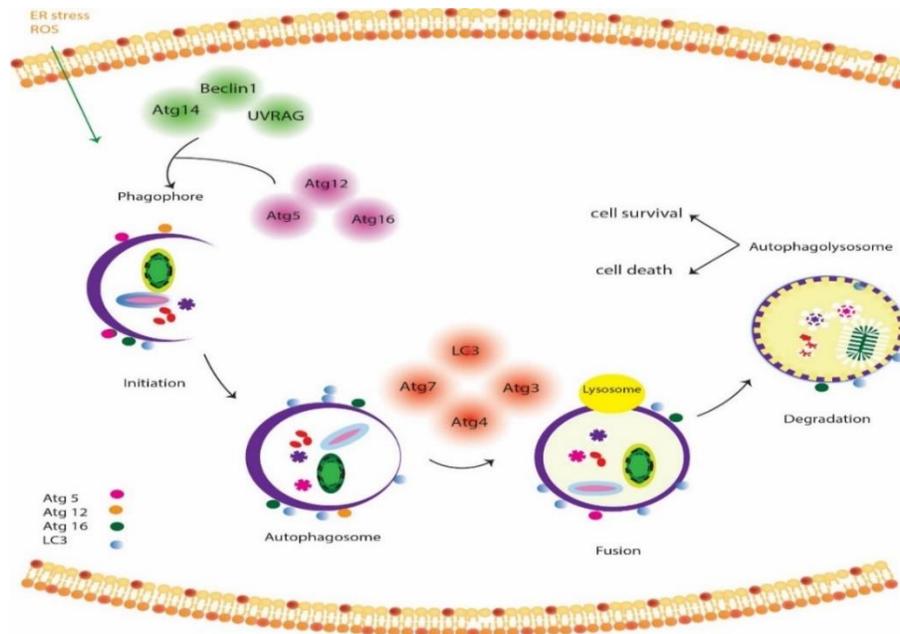


Figure 2. Autophagy signaling pathway regulation. The autophagy process is regulated via Autophagy-related genes (Atg) and their homologs in various eukaryotic cells. Autophagosome formation: Autophagy start with making phagophore assembly sites. Initiation of the phagophore structure belong to activity of Class-III PI3K Vps34. Vps34 is a protein complex which including Atg6 (Beclin1), Atg14 and Vps15 proteins. In addition to PI3KIII Vps34, other Atg family proteins (Atg5, Atg12, Atg16) are also have role in the organization of the phagophore structure.

Autophagosome elongation: in the first reaction Atg5 and Atg12 have important role and they are conjugated each other in presence of Atg7 and Atg10. The formation of this conjugation needs Vps34 function. Phagophores are prolonged by Atg5/Atg12 associated membranes which are targeted by LC3. Study of LC3-I to LC3-II conversion results in autophagosome formation. Autophagosome maturation and fusion: lysosomes from the cytoplasm transferred to the autophagosome

mTOR/ PI3K/Akt/

The phosphoinositide 3-kinase (PI3K), AKT (also known as protein kinase B), and mammalian target of rapamycin (mTOR) are described as a signaling pathway in most cancers. This signaling network controls cell cycle, metabolism, genomic instability survival²⁴, migration adhesion, especially during cancer progression, radioresistance, and metastasis^{25,26}. MTOR is a main evolutionarily conserved member in the autophagy network. MTOR contained two complexes, mTORC1 and mTORC2. Class I PI3K enzymes are able to phosphorylate PtdIns4P and PtdIns^{4,5} P₂ to create PtdIns^{3,4} P₂ and PtdIns^{3,4,5} P₃ that bind to AKT. The kinase AKT is necessary for activating mTORC2 through GTPase-activating proteins, tuberous sclerosis complex 1&2 (TSC1&2), and Ras homolog enriched in brain (Rheb). Activation of this pathway has an inhibitory effect on autophagy. Growth factors induce the class I PI3K/Akt/mTOR signaling pathway which leads to the inhibition of autophagy. In the lack of growth factors, cells are incapable of taking up nutrients from the extracellular medium. The inhibition of mTOR due to lack of growth factor consequently activates the autophagy machinery which leads to rescue cells from death via preserving ATP levels in starved cells⁶.

FOXO

Forkhead Box (FOX) O, characterized by a winged-helix domain- transcription factors, plays a fundamental role in maintaining cellular redox homeostasis by activating genes involved in free radical scavenging and apoptosis. Provided a role for PI3K-Akt-mTOR signaling in regulating autophagy has been comprehended for some years, a role for FOXO transcription factors has only recently become clear. However, several studies have shown evidence that these transcription factors may act as fundamental regulators of autophagy²⁷.

Bcl-2 family

Beclin 1(mammalian ortholog of yeast Atg6/Vps30)²⁸ is a haploinsufficient tumor suppressor gene which is mostly monoallelically deleted in many human cancers, including ovarian, breast, and prostate. Beclin 1 cooperates with many autophagy proteins

to initiate and maintain the autophagy pathway. This protein interacts with class III PI3K to provide autophagosome formation²⁹. In addition, Beclin 1 has a direct association with Bcl-2 family³⁰. The Bcl-2 family inhibits apoptosis through binding the proapoptotic effector proteins Bak, Bax, and also BH3-only proteins³¹. The interaction between the autophagy protein (Beclin 1) and the antiapoptotic protein (Bcl-2) indicates a potentially main point of connection between apoptotic and autophagic machinery²¹. Scientists indicate the dual function of Bcl-2 family in autophagy regulation. Proapoptotic proteins such as BH3 proteins can enhance autophagy, whereas Bcl-2 family such as Bcl-2, Bcl-w, and Bcl-XL as antiapoptotic proteins can prevent autophagy^{21,32}.

ROS

Different levels of ROS can initiate signals in the various signaling pathways such as autophagy. Many studies demonstrated that starvation can enhance the production of ROS, and this could lead to autophagy induction.

P53

P53, known as a mutated gene in human cancers, also modulate mTOR activity. Nuclear P53 promotes translation of autophagy through activated AMPK, while cytosolic P53 inhibits autophagy by phosphorylation of AMPK³³. The mitogen-activated protein kinase (MAPK) process regulates the maturation of autophagosomes. The role of AMPK in the maturation of autophagosome and lysosome fusion is not completely clear. AMPK can negatively regulate the mTORC1 pathway, and mTORC1 activation can suppress maturation of autophagosome via UVRAG phosphorylation^{34,35}.

Survival or death

Autophagy has been known as a necessary cellular process which requires for regulation of tumor progression and development. It also determines the sensitivity of cancer cells to anticancer therapy as well. However, the role of autophagy in tumor cells is complicated and depending on the conditions and have opposite roles in tumor proliferation. Both

autophagic cell survival and autophagic cell death have been seen in many cancers³⁶.

Autophagy can be activated in tumor cells (the same as normal cells) through stress condition such as hypoxia and starvation³⁷. This kind of environmental stress combines with intrinsic metabolic stress derived from the high metabolic requirement for metabolism and cell proliferation leading to insufficient ATP production. Autophagy is essential for tumor cells to survive metabolic stress. Genetic inhibition of autophagy inhibits survival in response to metabolic deprivation when apoptosis is inactivated^{38, 39}. Malignant cell activates autophagy in response to stress, providing long-term survival, especially in defective apoptosis. As autophagy is a survival mechanism, it is likely to cause survival of established cancer cells under various stress conditions⁴⁰. Tumor cells mostly encounter defects in apoptosis which lead to permitting autophagy to keep survival by optimizing nutrient utilization, metabolic stress, or degradation of organelles^{37,41}. In poorly vascularized tumors, most cells encounter hypoxia and metabolic stress leading to activation of the autophagy signaling pathway⁴² which can give an advantage to cancer cells to promote invasion and drug resistance.

In addition to cell survival and cell proliferation, autophagy in some conditions acts as a programmed cell death (called programmed cell death type II⁴³) as well as in tumor suppression⁴⁴. Different studies demonstrated that autophagic cell death is activated in tumor cells including colon⁴⁵, breast⁴⁶, and prostate⁴⁷ in response to different anticancer drugs. In addition, autophagy defects have been seen in different human tumors. Allelic loss of the required autophagy gene, beclin 1, is associated with human ovarian, breast, and prostate cancer^{37,48}. Furthermore, the loss of other autophagy regulators, including Atg5^{49,50}, Atg4C⁵¹, and Bif-1 are also found³⁷.

Autophagy inhibitor

Various studies indicate that chemical inhibition or genetic knockdown of autophagy can significantly enhance cancer cell death which is induced via anti-tumor medicine in preclinical models^{10, 52}.

The initial phase of triggering autophagy is membrane nucleation which is regulated through Beclin1 and ULK complex. Inhibitors of Beclin1 and ULK complex have been proven to block autophagy. These inhibitors inhibit MAP kinases, ERK, p38, and JNK1. For vesicle expansion and formation, the activation of Atg and LC3 protein is necessary, and inhibition of PI3K class III can block autophagy. Pharmacological autophagy inhibitors can be categorized as early or late step inhibitors of the pathway. Early phase inhibitors include 3-methyladenine, LY294002, and wortmannin which target the class III PI3K (Vps34) and interrupt the recruitment of membranes. Late phase inhibitors include hydroxychloroquine, chloroquine, monensin and Bafilomycin A1¹⁰. Chloroquine leads to minor acidic conditions and then decrease lysosomal function⁵³. Bafilomycin A1 is a particular inhibitor of the vacuolar type H⁺-ATPase (V-ATPase) in cells and prevent the acidification of organelles containing this enzyme⁵⁴.

Inhibition of autophagy in apoptosis-defective colon cancer and leukemia cell lines has been shown to sensitize resistant cells to TRAIL-mediated apoptosis⁵⁵.

Autophagy activator

In addition to autophagy inhibitors, autophagy activator indicates an antitumor effect. The autophagy activator is used as a remedy that causes cancer cells to encounter autophagy cell death. The activation of autophagy in defective apoptosis could enhance autophagic cell death⁵⁶ to overcome cancer. Special conditions and medicines in clinical use have been reported to increase autophagy.

The result of studies indicates that the physiologically complete privation of amino acid induces autophagy pathway⁵⁷. Misfolded or unfolded protein accumulation in the endoplasmic reticulum (ER) leads to ER stress, a mechanism in which macromolecules are recycled in eukaryotic cells. Researchers indicate that ER stress is a strong activator of autophagy⁵⁸⁻⁶⁰. In addition, some chemical compounds can induce autophagy, including Everolimus (RAD001), Rapamycin, Temsirolimus (CCI-779), Deforolimus (AP-23573), etc.

some of these activators inhibit mTOR which activate autophagy in a different type of malignancies, including acute myeloid leukemia (AML)⁵. Rapamycin as an inhibitor blocks mTOR activity in vivo and also sensitizes tumors to apoptosis induced through chemotherapy⁶¹. Autophagy inhibitors and

autophagy activators facilitate autophagy research and consequently facilitate its therapeutic potential in human diseases. As most autophagy inhibitors are not quite specific, genetic intervention can be a good choice to block autophagy, including RNAi and miR against Atg genes⁵⁷.

Table 1. Autophagy inhibitors/activators

Autophagy inhibitor	Mechanism	Reference
3-Methyladenine	inhibits autophagy via blocking autophagosome formation through inhibition of class III PI3K	(42, 90, 91)
LY294002	PI 3-kinase inhibitor	(57, 92, 93)
Wortmaninn	PI 3-kinase inhibitor	(57, 92)
Hydroxychloroquine	Change degradation step of autophagy by limiting acidification of lysosomes	(94-96)
Chloroquine	inhibits autophagy via raising the lysosomal pH	(97, 98)
Monensin	Change degradation step of autophagy by limiting acidification of lysosomes	(99)
Bafilomycin A1	Inhibit Autophagolysosome formation by inhibiting Vacuolar-type H(+)-ATPase	(54, 100)
Autophagy activator Everolimus (RAD001)	Activate autophagy through inhibiting mTOR and Down regulating AKT signaling	(101-104)
Rapamycin	Activate autophagy through inhibiting mTOR	(105)
Temsirolimus (CCI-779)	Activate autophagy through inhibiting mTOR and Down regulating AKT signaling	(103)
Deforolimus (AP-23573)	Activate autophagy through inhibiting mTOR	(103)
Brefeldin A	Activate autophagy via inducing ER stress	(106)
Thapsigargin	Activate autophagy via inducing ER stress	(106)
Tunicamycin	Activate autophagy via inducing ER stress	(106)

AML

Approximately ten percent of all cancer mortalities result from leukemia and lymphomas in the Western world⁶². Improvements in lifestyle and health care have increased the overall average lifespan, whereas AML will be an increasing problem worldwide⁶². Acute leukemia is a highly heterogeneous class of malignant hematopoietic disorders which characterized by high proliferation of clonal neoplastic cells belonging to either the myeloid or lymphoid lineage^{13,63}. AML is a phenotypically and genetically heterogeneous disease. It has been known as an aggressive disorder with high variability in clinical outcome. Different studies have been focused to determine the mechanisms of leukemogenesis in AML. The LSC comes from cumulating of gene mutation, including P53, NPM1 (apoptosis and cell cycle regulation), AML1, CEBPA (myeloid differentiation) and FLT3, RAS, c-KIT, protein tyrosine standard phosphatase non-receptor 11 (PTPN11) through the effect on cell proliferation⁶⁴.

Mutations in upstream receptors such as FLT3, KIT and granulocyte colony-stimulating factor receptor have been found in AML which may induce activation of the JAK/STAT, Ras/Raf/MEK/ERK, and PI3K/PTEN/Akt/mTOR signaling network that leads to inhibition of apoptosis⁶⁵. In addition, upregulation of vascular endothelial growth factor receptor (VEGF-R) has been documented in AML, which can cause the activation of these pathways⁶². Furthermore, overexpression of PI3K/PTEN/Akt/mTOR and Ras/Raf/MEK/ERK are frequently observed in AML patient⁶².

Autophagy and the hematopoietic niche

The main question is how stem cells adjust their self-renewal and multipotency properties. Stem cells miss the potential for continued self-renewal when removed from their normal cellular environment, called stem cell niche, suggesting a vital role for the microenvironment in regulating stem cell behavior⁶⁶. The two principal components of the hematopoietic niche are HSCs and mesenchymal stem cells. HSCs reside and self-renew in two distinct BM niches: the 'osteoblastic (endosteal)' and 'vascular' niches⁶⁷. The BM niche adjusts HSC quiescence,

differentiation, proliferation, and migration. HSCs interact with the niche through different molecular signals, including adhesion mechanisms^{66, 67}. The perfect adjustment of HSC quiescence and self-renewal is not fully clear. LSCs Like normal HSCs associated with signals from the hematopoiesis-regulating stromal environment for proliferation and survival. LSCs represent the unique characteristics as stem cells, including quiescence, pluripotency and self-renewal through the bone marrow microenvironment. Although the biology of LSCs shares many similarities with that of HSCs, LSCs can destroy HSCs, hijacking the bone marrow microenvironment. BM niches potentiate the growth of leukemia cell survival and drug resistance by providing the essential cytokines and cell contact-mediated signals. On the other hand, LSCs themselves change the BM stromal cell composition or function that result in the survival of the leukemic cells⁶⁷.

Autophagy, a general cellular housekeeping process, is essential for differentiation and self-renewal of adult human stem cells, which mainly contribute to cytotoxic drug resistance. Meanwhile, a long-known phenomenon of these cells are sensitive against differentiation or blockage of autophagy⁶⁶. Recent studies demonstrated that mechanisms of autophagy are active in HSC. For example, FIP200 is essential not only for the induction of autophagy but also for the maintenance role of HSC in vivo. Furthermore, HSC lacking ATG 7, another key autophagy protein, was also impotent to survive under in vivo situation⁶⁶. Autophagy seems crucial for the equilibrium between quiescence, self-renewal and differentiation of mesenchymal stem cells^{68,69}. FOXO transcription factors play an essential role in HSC maintenance which has been proved in original research by the conditional deletion of Foxo1, Foxo3a, and Foxo4 in the adult hematopoietic system resulting in lymphoid developmental abnormalities, myeloid lineage expansion, and a significant decrease of HSCs. At first, HSCs exhibited an effective long-term repopulating activity by downregulation of proteins involved in the cell cycle by FOXO, including cyclin G2, p130/Rb, p27, and p21⁷⁰.

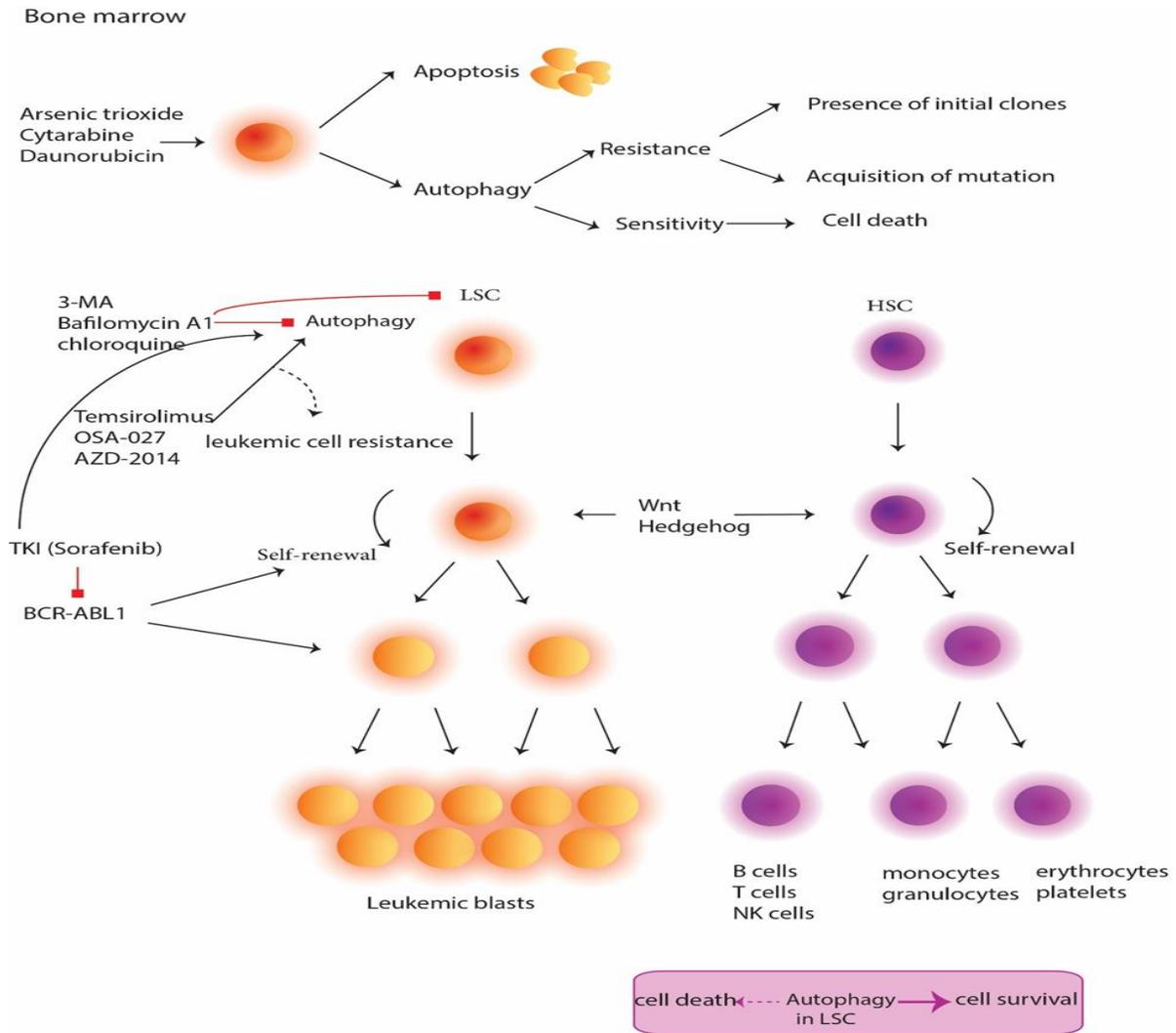


Figure 3. The bone marrow niche adjusts HSC quiescence, differentiation, proliferation, and migration. HSCs interact with the niche through different molecular signals. Leukemic blasts originate from common primary progenitor cells that have the capacity to pluripotency and self-renewal through the bone marrow microenvironment. Conventional therapy for AML has been planned to eliminate leukaemic blasts. BM niches potentiate the growth of leukemia cells and which consequently leads to drug resistance and metastatic of leukaemic cells via shielding LSCs.

Role of autophagy in AML

Various studies have shown that autophagy acts as a double-edged sword in the treatment of cancer. From one side, autophagy can promote maintenance of cancer cells, same as leukemia stem cells, or even play a role as a drug resistance mechanism enhancing cancer cell survival through self-digestion. On the other side, the mechanism of autophagy can improve tumor suppressive by effective antitumor immunity while support healthy tissues from the toxicity of anticancer therapies⁵. Some mutations in genes affecting autophagy include beclin-1, PI3K, Akt, p53, and Bcl-2, having a role in the pathogenesis of malignant lymphoma, breast, prostate and ovarian cancer⁷¹. PI3K/Akt signaling pathway is activated in various cancer types and is a vital event in tumorigenesis⁷². Autophagy incriminates as the main mechanism through some antileukemic compounds. Dysregulation of the PI3K/Akt/mTOR signaling pathway resulted in increasing chemotherapy resistance via inhibition of apoptosis, leukemic cell survival, and induce cap-dependent translation of mRNAs essential for differentiation, cell cycle progression, and growth. Initiation of this network is a common feature of leukemia and can dictate poor prognosis⁷³. Therefore, it is reasonable to consider that targeting mTOR through rapamycin can effectively help to treat tumors. Rapamycin derivatives potentially disrupt AKT activity in leukemic cells through^{74, 75} suppression of mTORC2 and mTORC1 pathway⁷⁶. The fundamental action of the mTORC1 pathway has been discovered in many primary AML samples. In most AMLs cases, mTORC1 activation may be caused by an autocrine insulin-like growth factor-1⁷⁴.

Alternative therapy for leukemia such as dasatinib⁷⁷, arsenic trioxide⁷⁸, vitamin D3⁷⁹, histone deacetylase inhibitors⁸⁰, and platonin inhibitor⁸¹ induce autophagy as their mechanism of action. For instance, Histone deacetylase inhibitors can trigger apoptosis in myeloid leukemia blasts by suppressing autophagy, while platonin trigger autophagy programmed cell death in human leukemia cell lines. Chemotherapeutic drugs in the anti-AML treatment, induce autophagy, including daunorubicin, idarubicin, cytarabine, vitamin D3, and Arsenic

trioxide¹¹. The induction of autophagy has recently been implicated in the regulation of leukemic cell death triggered by anticancer drugs¹⁴. As mentioned, autophagy can play as a tumor suppressor or cell survival in cancer cells. Hence, when autophagy acts as a cell survival, there is a potent rationale for the use of autophagy blockers in cancer treatment strategy⁵.

Anthracycline daunorubicin

Anthracycline daunorubicin is one of the main antitumor agents broadly used in the treatment of myeloid leukemia. Unfortunately, the clinical efficacy of daunorubicin was limited because of its cardiac toxicity, renal toxicity, and severe myelosuppression with high-dose chemotherapy. A group of anticancer compounds such as DNA-damaging chemotherapeutic agents have been indicated to increase the accumulation of autophagosomes in tumor cell lines. In a research, scientists indicated that daunorubicin induces autophagy via activation of MEK1/2 and ERK1/2 in myeloid leukemia cells. Inhibition of autophagy via autophagy inhibitors (chloroquine or Atg5/Atg7 knockdown) or inhibitors of MAPK increased the activation of caspases and tumor inhibition which induced by daunorubicin⁸².

Idarubicin

Idarubicin is an anthracycline antileukemic agent widely used for the treatment of AML, chronic myelogenous leukemia, acute lymphoblastic leukemia and myelodysplastic syndromes. Scientists in an investigation reported that idarubicin induces autophagy in leukemia cell lines and primary leukemic cell through mTOR repression. Furthermore, they observed inhibition of Akt by Idarubicin in leukemic cells. Repression of mTOR activity by idarubicin may partly due to the blockage of Akt since the Akt is one of the most leading activators of mTOR⁸³.

Cytarabine

Cytarabine is another chemotherapeutic agent that used alone or in combination with other compounds to treat different forms of leukemia. In a study,

researchers indicated that cytarabine induces autophagy in leukemic cell lines through suppression of mTOR. In addition, they showed that inhibition of autophagy by either pharmacological (Chloroquine) or genetic inhibitors enhance apoptosis in cytarabine-treated leukemic cells⁸⁴.

Vitamin D3

Vitamin D3 plays vital roles in regulating physiological and cellular responses. Vitamin D3 potently inhibits cell proliferation in various cancer cells such as myeloid leukemia. It inhibits angiogenesis, tumor invasion, and metastases. Scientists demonstrated that vitamin D3 is able to induce autophagy through up-regulating beclin1, and they indicated that eliminating beclin1 (beclin 1 act as an antiapoptotic role during vitamin D3-induced differentiation of the HL60 cell line) suppress autophagy and induce apoptosis⁷⁹.

Arsenic trioxide

Arsenic trioxide has been reported to have strong antitumor effects *in vitro* and *in vivo* by increasing cell death, cell cycle arrest, and apoptosis in leukemia cells. It has the main clinical activity in the treatment of patients with acute promyelocytic leukemia (a subtype of AML)⁸⁵. In addition to apoptosis, arsenic trioxide induce autophagy⁸⁶ through up-regulation of beclin1⁸⁷.

Moreover, autophagy inhibitors have anti-angiogenic features owing to their interference with endothelial cell survival⁸⁸. As the role of bone marrow angiogenesis in human leukemia has been demonstrated⁸⁹, another mechanism through which autophagy inhibitors could be beneficial is by affecting cancer cell vasculature⁵.

CONCLUSION

Autophagy is a self-degradative signaling pathway which has the main role in balancing of energy at crucial times in response to nutrient stress and development. Autophagy includes some sequential steps, including induction, insertion to lysosomes, degradation, and utilization of degradation products, and each step may perform a different function⁹. Many studies of autophagy confirmed that this process of proteolysis has a function in the

regulation of cell survival and death. Conventional regimen (daunorubicin, idarubicin and cytarabine) used for the treatment of AML which induced autophagy¹⁷. Hence, it is serious to know the function of autophagy which began with these agents. Recently, scientists use the autophagy inducers or autophagy inhibitors in combination with conventional medicine to promote the new strategy for cancer remedy. However, the function of autophagy during leukemia treatment is not clear completely¹⁸.

As findings discussed in this review, it is obvious that autophagy is affected by various therapeutic treatments used for AML. Autophagy functions are not always identical. Sometimes this signaling pathway protects leukemic cells against cancer therapy, whereas sometimes it is essential for the remedy to be efficacious. Autophagy modulation, both stimulatory and repressive, can be attained by participating with different signaling pathways at multiple levels.

The regulation of autophagy is complicated and includes many signaling pathways. Therefore, the safety and effectiveness of autophagy inhibitors and activators must be studied completely before clinical therapeutic development.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

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