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Role of Autophagy and Apoptosis in Acute Lymphoblastic Leukemia

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Abstract

Background: Acute lymphoblastic leukemia (ALL) is a malignant disease characterized by an excessive number of immature lymphocytes, including immature precursors of both B- and T cells. ALL affects children more often than adults. Immature lymphocytes lead to arrested differentiation and proliferation of cells. Its conventional treatments involve medication with dexamethasone, vincristine, and other anticancer drugs. Although the current first-line drugs can achieve effective treatment, they still cannot prevent the recurrence of some patients with ALL. Treatments have high risk of recurrence especially after the first remission. Currently, novel therapies to treat ALL are in need. Autophagy and apoptosis play important roles in regulating cancer development. Autophagy involves degradation of proteins and organelles, and apoptosis leads to cell death. These phenomena are crucial in cancer progression. Past studies reported that many potential anticancer agents regulate intracellular signaling pathways.

Methods: The authors discuss the recent research findings on the role of autophagy and apoptosis in ALL.

Results: The autophagy and apoptosis are widely used in the treatment of ALL. Most studies showed that many agents regulate autophagy and apoptosis in ALL cell models, clinical trials, and ALL animal models.

Conclusions: In summary, activating autophagy and apoptosis pathways are the main strategies for ALL treatments. For ALL, combining new drugs with traditional chemotherapy and glucocorticoids treatments can achieve the greatest therapeutic effect by activating autophagy and apoptosis.

Keywords

acute lymphoblastic leukemia, autophagy, apoptosis

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Introduction

Acute lymphoblastic leukemia (ALL) is a hematological malignant disease characterized by over numbered immature lymphocytes, of which 80-85% are B cells and 20-25% are T cells. The result is arrested differentiation and abnormal proliferation of these lymphocytes.[1,2] Acute leukemia is the most common form of cancer in children, comprising ~30% of all childhood malignancies. Of the acute leukemias, acute lymphoblastic leukemia (ALL) occurs 5 times more often than acute myeloid leukemia (AML). In 2020, the worldwide incidence of ALL in population is estimated between 0.4 to 2 per 100,000, and prevalence rate between 0.37 to 1.6 per 100,000. ALL most commonly occurs in children, but it is also diagnosed in adults. Its peak incidence is between 2 to 5 years of age, and beyond that, 60% of cases occur prior to the age of 20. Incidence rates show no gender differences.[3,4] Although the

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5-year survival rate of ALL is about 90%, 20% of children with ALL relapse with poor prognosis.[5,6] Adult patients of ALL have recurrence rates comparatively higher than children, up to 40 or 50%.[7,8] As a result, efforts are currently made to better treat ALL. Children with ALL have poor social, physical, and emotional health performances compared with normal children and siblings of the same age. In addition, ALL children also develop problems of depression, anxiety, and attentional disorders.[9,10] Current treatments of ALL include chemotherapy, with high doses of methotrexate (MTX), 6-Mercaptopurine (6MP) and other drugs,[11-14] followed by regular oral or injection of anticancer drugs like Dexamethasone, Vincristine, cytarabine (Ara-C), Endoxan, 6MP, and MTX. However, ALL sometimes recurs, likely due to residual cancer cells escaping treatment. These cells, when proliferated, will result in the reappearance of the disease.[15] When cancer thus returns, it is called relapse or recurrence. That is to say, blast cells are present in bone marrow after reaching complete remission. About 15-20% of childhood ALL patients relapse.[16] Therefore, how to effectively detect minimal residual disease (MRD) is important for the prognosis of ALL. MRD is assessed through the examination of remission bone marrow samples. There are several common methods of MRD assessment, such as polymerase chain reaction, flow cytometry and next-generation sequencing.[17] In addition to detecting MRD, from a molecular perspective, apoptosis and autophagy are extremely important aspects in treating relapse of ALL. The regulation of apoptosis is known to be abnormal in relapse ALL. Relapse in childhood ALL is associated with a drop in the Bax/Bcl-2 ratio, and loss of spontaneous caspase-3 processing *in vivo.*[18] Genes of the Bcl-2 family are responsible for controlling pro-apoptotic and anti-apoptotic pathways. In addition, impairments of PI3K/Akt/mTORC1 and Notch1 signaling pathways are found in ALL, affecting the regulation of autophagy.[19] However, the controversial role of autophagy in promoting or inhibiting leukemia still needs to be clarified. Future medical treatments shall aim to lower recurrences of ALL patients, especially those after their first remission.

Autophagy and B-cell Acute Lymphoblastic Leukemia (B-ALL)

Autophagy is a process that, through intracellular lysosomes, eliminates old proteins, abnormal organelles or foreign invading microorganisms. Autophagy plays an important role in clearing old organelles and reducing oxidative stress to maintain cell health, avoiding oxidative stress,[20,21] and consequently minimizing cancer development. B-cell acute lymphoblastic leukemia (B-ALL) is treated with glucocorticoids that induce cell autophagy and cause cell death.[22,23] In B-ALL, ETV6-RUNX1 played an important role on inducing genetic change and leading to tumorgenesis.[24]ETV6-RUNX1 fusion protein, which plays a role in the development of B-ALL, accounts for 25% of pediatric patients with B-cell precursor acute lymphoblastic leukemia (BCP-ALL). In ETV6-RUNX1-positive cells, autophagy activates cell proliferation, survival and drug resistance. The expression of ETV6-RUNX1 could be regulated by autophagy-regulating complex such as Vps34, Beclin-1, and Vps15.[25] In the recent study demonstrated that through suppressing autophagy in ETV6-RXNX1 gene positive B-ALL could severely downregulate cell proliferation and survival.[26] Hydroxychloroquine, an autophagy inhibitor, reduces proliferation and survival of leukemic blasts in BCP-ALL.[26] Furthermore, the ongoing clinical trials also showed the same strategy to against B-ALL proliferation. For example, an autophagy inhibitor, chloroauine, could increase the response of patients with B-ALL to the chemotherapy.[27] These studies supported the rationale between autophagy inhibition and B-ALL suppression and showed the promising treatment effects. For B-ALL, GC resistance is a key predictor of the adverse outcome during the initial stage of chemotherapy. ALL with GC-resistance shows higher expressions of the MAPK pathway.[28-30] The development of inhibitors, which target components of MAPK pathway, attracts great research interest. Selumetinib, a MEK1/2 inhibitor, enhances dexamethasone toxicity, reduces pERK1/2 level and mTOR signaling pathways.[29,30] Besides, selumetinib treatment also up-regulates a specific marker of autophagy LC3-ll level.[29] Down-regulation of autophagy is closely related to B-ALL. The Atg7 gene is one player of the autophagy pathway. Atg7 acts as an E1-like enzyme that conjugates itself with Atg12 and Atg5. This conjugated complex is important for driving phosphatidylethanolamine (PE) to LC3. Atg7 activates the conjugation of LC3 with PE during autophagy.[31] Deletion of Atg7, by conditional knockout in B-ALL xenograft mouse model, produces Atg7 deficient mice which are more susceptible to the occurrence of engrafted human leukemia cells.[32] Bone marrow cells of pediatric B-ALL patients display lower levels of expressing autophagy genes, like Beclin-1, Atg5, Atg7, LC3 and p62. When autophagy is activated by rapamycin, leukemia bone marrow cell cycle arrest is inhibited, and thereby improving the survival of ALL xenograft mice. Furthermore, this study found that autophagy, collaborating with ubiquitination, could downregulate oncoprotein in pediatric B-ALL.[33]

Autophagy and T-Cell Acute Lymphoblastic Leukemia (T-ALL)

T-ALL is a common pediatric malignancy, comprising 20-25% of ALL. In the ALL Jurkat cell model, timosaponin A III induces cell autophagy and apoptosis to exert its antitumor effects.[34] The JAK-STAT pathway cascade regulates lymphoid cells in their formation, proliferation, survival and differentiation. Studies on leukemia reported that the JAK-STAT pathway is frequently mutated. TG101209, a JAK2 inhibitor, inhibits T-ALL cell proliferation by regulating JAK-STAT pathway and autophagy.[35] Autophagy may play an important role in cytotoxic effects of Akt inhibitors in protecting T-ALL cells. C Simioni et al, used MK-2206 in combination with an autophagy inhibitor, either bafilomycin A1 or

Table 1. Autophagy-Inducing/Inhibiting Agents in ALL Treatments.

Disease	Cell type/clinical trial/ animal model	Treatment	Mechanism	Inhibit/induce autophagy	Ref
B-ALL	GC-resistant B-ALL cells	Selumetinib	Inhibition of mTOR signaling pathway, inhibition of MEK/ERK pathway	Induce	29
B-ALL	pre-B ALL cell lines	Dexamethasone	Accumulation of autophagosomes, increase LC3-II accumulation	Induce	54
T-ALL	CCRF-CEM	Dexamethasone	Suppression of glycolysis and activation of mitochondrial function	Induce	55
T-ALL	Jurkat	Tamoxifen	In a G protein-coupled ER-dependent manner	Induce	56
B-ALL	SUP-B15	Curcumin	Activation of RAF/MEK/ERK pathway	Induce	47
ALL	Clinical trial	Obatoclax	Increased LC3-I to LC3-II conversion	Induce	57
ALL	ALL xenograft mice	Berberine	Induced autophagy via inactivating AKT/mTORC1 signaling pathway	Induce	58
ALL	ALL xenograft mice	RAD001 (Everolimus)	Increased in the autophagy-associated protein Beclin-1 and the processing of LC3 to the lipidated form (LC3-II), which associates with autophagosomes	Induce	59
B-ALL	ALL xenograft mice	Alantolactone	Induced apoptosis and inhibited autophagy of ALL cells via upregulation of adaptor related protein complex 2 subunit mu 1 (AP2M1)	Inhibit	60

Abbreviations: B-ALL, B-cell acute lymphoblastic leukemia; T-ALL, T-cell acute lymphoblastic leukemia.

chloroquine, to increase cytotoxicity. MK-2206 can therefore induce autophagy in T-ALL cells, protecting tumor cells against apoptosis.[36] The anti-malarial drug chloroquine (CQ), an autophagy inhibitor, affects oncogenic NOTCH1 trafficking and processing in T-ALL. Besides, CQ also induces apoptosis and inhibits T-ALL cell proliferation.[37] Targeting mutated NOTCH1 proteins could increase anti-leukemia activity in the T-ALL xenograft mouse model.[38] The autophagy-related proteins such as LC3-II, Atg5, Beclin-1 are key markers. The 20(S)-ginsenoside Rh2 (GRh2) is a bioactive compound isolated from ginseng, which has beneficial effects on anti-cancer. After 20(S)-ginsenoside Rh2 (GRh2) treatment in T-ALL, not only the autophagic flux is enhanced, but also levels of LC3-II, Atg5, Beclin-1 are upregulated.[39,40] Also 20(S)-ginsenoside Rh2 (GRh2) down-regulates levels of CD3 and CD45 in the bone marrow of T-ALL xenograft mice. In addition, 20(S)-ginsenoside Rh2 (GRh2) also regulates PI3K/Akt/mTOR signaling pathway.[41] In general, cancer cell development need overcome the energy consumption and oxygen supplement, therefore, autophagy played the major role on providing more ATP to cancer cell and defeating hypoxic stress. Therefore, targeting inhibiting T-ALL autophagy provided a promising strategy to increase treatment efficacy or sensitivity to the chemotherapy.[42] Recombinant human arginase (rhArg) has been demonstrated that could effectively decrease hepatocellular carcinoma proliferation in the clinical trials.[43] In the recent year, the effects of rhArg on treating T-ALL has also been investigated. In *in vitro* experiments showed that rhArg induced both autophagy and apoptosis in the T-ALL cell lines. However, by treated autophagy inhibitor and rhArg at the same time, which could significantly enhance the rhArg induced cell apoptosis.[44] Furthermore, NL-101 compound which inhibited T-ALL autophagy has been reported that could suppress T-ALL proliferation through inducing cell cycle arrest and cell apoptosis.[45] These studies suggested that targeting suppressing autophagy in T-ALL was effective and could be used to support further clinical trials in the future.

Autophagy and Philadelphia Chromosome-Positive ALL

The Philadelphia chromosome-positive ALL has a t(9;22)(q34;q11) translocation in the Philadelphia chromosome.[46] In Philadelphia chromosome-positive ALL, which is the most frequent genetic aberration in ALL, different strategies are used to induce autophagy. In this disease, the curcumin-induced autophagy via the ERK1/2 pathway.[47] On the other hand, tyrosine kinase inhibitors (TKIs) also show good effects in its treatment. The combination of BCR-ABL1 inhibitors (TKIs) and PI3K/Akt/mTOR inhibitors could effectively induce apoptosis and autophagy, leading to lower viability of T-ALL cells.[48] PI3K played an important role in regulating cell proliferation, differentiation, survival, cell cycle and metabolism in leukemia.[49,50] Furthermore, recent study indicate that PI3K inhibitors combine with TKIs induce autophagy in Philadelphia chromosome-positive B-ALL cell lines.[51] In the recent years, it has been proven that metformin could reduce the incidence of cancer and improve the outcome of chemotherapy.[52] In *in vitro* study, metformin could induce apoptosis cell death in Philadelphia chromosome-positive ALL by activating AMP-activated protein kinase (AMPK) and inhibiting mammalian target of rapamycin complex 1 (mTORC1) pathway. Besides, metformin also could induce autophagy through the ERK signaling pathway in Philadelphia chromosome-positive ALL cell line.[53] Therefore, these studies suggested that the combination TKIs with PI3K/Akt/mTOR inhibitors can be used as a novel therapeutic approach for Philadelphia chromosome-positive ALL in future clinical application.

Autophagy is widely used in treating ALL, as summarized in Table 1.

Apoptosis and ALL

Programmed cell death, or apoptosis, is a process generally characterized by distinct morphological changes. It is mediated through energy-dependent biochemical mechanisms. Relapse

Table 2. Apoptosis-Inducing Agents in ALL Treatments.

Disease	Cell type/clinical trial/animal model	Treatment	Mechanism	Ref
T-ALL	CCRF-CEM, JURKAT and MOLT-4	CFTR-inh172	Inhibited cell proliferation, promoted apoptosis and arrested the cell cycle	89
B-ALL	pre-B ALL cell lines	Dexamethasone	Upregulation of promyelocytic leukemia protein	82
ALL		L-asparaginase	Activated inositol 1,4,5-trisphosphate (IP3)-induced Ca^{2+} signaling in a Huntingin-associated protein 1 (HAP1) dependent manner	90
T-ALL	Jurkat	Ginsenoside Rh2	Inhibited PI3K/AKT pathway	91
T-ALL	Jurkat	FHL1C	Suppressed downstream target genes such as Hes1 and c-Myc and PI3K/AKT and NF-κB of Notch signaling pathways	92
ALL	Clinical trial	Obatoclax	Activated caspase-3 activity by time- and dose-dependent manner	85
B-ALL	Clinical trial	Pentoxifylline	Upregulated apoptotic extrinsic pathway	93
B-ALL	Clinical trial	Calphostin C	Induced apoptosis was markedly suppressed by BAPTA/AM, a cell-permeable Ca^{2+} chelator as well as $NiC1_2$, an inhibitor of Ca^{2+}/Mg^{2+}-dependent endonucleases	94
B-ALL	xenograft models of hypodiploid B-ALL	Bcl-2 inhibitor	Antiproliferative effect of Bcl-2 inhibition accompanied by induction of apoptosis as shown by increased levels of cleaved PARP	95
B-ALL	xenograft models	Apatinib	Induced apoptosis through suppressing the vascular endothelial growth factor receptor 2 (VEGFR2) signaling pathway	96

Abbreviations: B-ALL, B-cell acute lymphoblastic leukemia; T-ALL, T-cell acute lymphoblastic leukemia.

in childhood ALL is associated *in vivo* with a lower Bax/Bcl-2 ratio, and a loss of spontaneous processing of caspase-3.[61,62] Therefore, tumor-inhibiting effects can be exerted mainly through apoptosis. Mitochondria plays a key role in a number of cellular pathways, including the apoptosis pathway, reactive oxygen species (ROS) production and cell death induction. Mitochondria is an important organelle, which regulates many cellular pathways in mammalian cells. The dysfunction of apoptosis is common in cancer. Therefore, mitochondria is a target for anticancer treatment. During apoptosis, the mitochondrial membrane is depolarized, leading to a drop in the membrane potential (MMP). The loss of MMP increases the permeability of its outer membrane. Consequently, the leakage of mitochondrial membrane releases mitochondrial apoptosis factors, including cytochrome c, apoptosis–inducing factor and endonuclease G, leading to the activation of caspase-cascade.[63-65] Regulating mitochondrial functions is a therapeutic strategy which stops oxidative phosphorylation and releases proapoptotic proteins, like cytochrome c, Bcl-2 family, Bak and Bax.[66] Bcl-2 family proteins, such as *BCL-2, BCL-XL, BAX* and *BAK* are important proteins responsible for regulating pro- and anti-apoptosis effects. Among them, Bcl-2 is the most important anti-apoptotic protein and Bax is a pro-apoptotic protein. Remission failure of acute leukemia cases are closely related to high Bcl-2 / Bax ratios. This Bcl-2 / Bax ratio is an important parameter in ALL. When comparing ALL patients and healthy controls, polymorphism within the Bcl-2 promoter region is more reliable than that within the Bax promoter region for estimating the survival time of ALL patients.[67] *In vitro* study on CCRF-CEM cell line, which is an ALL chemotherapy-resistant model, high doses of prednisolone increases *Bax* gene expression but decreases *Bcl-2* gene expression. Therefore, prednisolone activates the apoptosis pathway by up-regulating Bax expression and down-regulating Bcl-2 expression.[68] The Bcl-2 family represents an important regulator in the intrinsic apoptosis pathway. Bcl-2 protein family overexpression is one chemo-resistance mechanism. Previous studies targeted the Bcl-2 pathway to treat ALL. BH3 mimetic venetoclax (ABT-199), a Bcl-2 inhibitor, could inhibit viability of human T-ALL cell lines, primary T-ALL samples, and *in vivo* xenograft mice.[69] Combining ABT-199 with chemotherapeutic agents showed synergic effects on treating ALL, including activated apoptosis markers such as caspase-3 and PARP.[70] ABT-737 is a small BH3 mimetic Bcl-2/Bcl-xL inhibitor. Treatment with ABT-737 has anti-leukemia activity and triggers mitochondrial apoptosis on ALL cells.[71] Mitochondria targeting is another anti-leukemia approach. Mitochondria regulates ROS which participates in various cellular signaling pathways such as cell cycle, differentiation, migration, proliferation and apoptosis. Mitochondria are the most important source of cellular ROS. ROS and apoptosis coordinate to maintain cellular homeostasis. Excessive ROS could induce apoptosis. Activation of ROS is thus a strategy for anti-leukemia. Matrine, an ingredient isolated from traditional Chinese medicinal herb, could effectively activate ROS production by a drop of MMP in ALL B-lymphocytes. Higher levels of Bax/Bcl-2 appear in ALL B-lymphocytes treated with matrine.[72] Matrine could induce apoptosis in ALL B-lymphocytes. For example, in treating B-cell ALL, bafilomycin A1 induces the binding of Beclin-1 to Bcl-2, and thereby promoting apoptosis and inhibiting autophagy and finally leading to cell deaths.[73] Histone deacetylases (HDACs) inhibitors induce the arrest of cell cycle and apoptosis, leading to halted cell proliferation. HDACs inhibitors are therefore widely used in leukemia treatment.[74]

Apoptosis and Glucocorticoid (GC)-Resistant ALL

In the past, the treatments of ALL have been chemotherapy and glucocorticoids (GCs). GCs show anti-leukemic activity by

first binding with glucocorticoid receptor (GR), and the activated GR binds to target genes in the nucleus, initiating the transcription processes.[75] GCs induce apoptosis via upregulation of pro-apoptotic or downregulation of anti-apoptotic genes. However, once ALL relapses, the resistance to GC becomes stronger. This situation is more commonly observed in pediatric T-ALL patients than in B-ALL patients.[76,77] To overcome this weakness, new agents are being developed to induce apoptosis in GC-resistant leukemic cells. For example, anisomycin can induce apoptosis GC-resistant T-ALL cells via activating cleaved caspase-3, mitogen-activated protein kinases (MAPKs) p38 and Jun N-terminal kinase (JNK).[78] Rapamycin plus dexamethasone induce more apoptosis and greater cell cycle arrest via inhibition of the PI3K/mTOR pathway.[79] In addition to activating the pro-apoptosis pathway, down-regulating the anti-apoptosis pathway is another approach to enhance apoptotic cell death. Ciclopirox olamine (CPX) has antileukemia effects by down-regulating anti-apoptotic proteins such as Bcl-2, Bcl-xL, and Mcl-1 in GC-resistant T-ALL cell lines.[80] Some studies used medicinal herbs to treat GC-resistant T-ALL. For example, tetrandrine (TET) and cepharanthine (CEP) could effectively induce apoptosis markers such as caspase-3, caspase-6, caspase-8, caspase-9, p53 and Bax in human leukemia Jurkat T cells. Besides, both TET and CEP not only upregulate apoptosis markers, but also downregulate mTOR and p-phosphatidylinositol 3-kinase.[81] Some studies have indicated that microRNAs (miRNAs) are related to the sensitivity to drugs, in particular GCs. Overexpressing miR-331-3p inhibits MAP2K7 levels, leading to reverse the GC resistance in GC-resistant CCRF-CEM cell line.[82] MAP2K7 has been reported to enhance cancer cell proliferation, metastasis and progression.[83,84] Also miR-124 is abnormally expressed in cancers. In ALL, miR-124 targets the GC receptor (NR3C1), leading to activation of GC resistance. Besides, miR-124 promotes proliferation and inhibits apoptosis in ALL cells. Therefore, targeting miR-124 can be a new therapeutic strategy in GC-resistant ALL patients.[85] Regulation of miR-17 family is associated with the sensitivity to dexamethasone. And the miR-17 family plays a crucial role in cell cycle, apoptosis, tumorigenesis and angiogenesis.[86-88] Table 2 summarizes previous studies showing the use of apoptosis-inducing agents in ALL treatments.

Conclusion

Studies on autophagy and apoptosis in ALL reported that enhanced activation of autophagy and apoptosis causes cell death in the human ALL cell line or primary ALL cells. Therefore, both autophagy and apoptosis have great potential for anticancer treatment for ALL. In summary, activating autophagy and apoptosis pathways are the main strategies for ALL treatments. Through the development of new drugs, combating relapsed ALL is an urgent medical goal. Traditional chemotherapy and glucocorticoids are no longer sufficient to deal with relapsed ALL. For ALL, combining new drugs with traditional chemotherapy and glucocorticoids treatments can achieve the greatest therapeutic effect by activating autophagy and apoptosis.

Authors' Note

No significant relationships exist between the authors and the companies/organizations whose products or services may be referenced in this article. Our study did not require an ethical board approval because it did not contain human or animal trials.

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