Redox Control in ALL

Subjects: Cell Biology

Contributor: Yongfeng Chen

Acute lymphoblastic leukemia (ALL) is a hematological malignancy originating from B- or T-lymphoid progenitor cells. Recent studies have shown that redox dysregulation caused by overproduction of reactive oxygen species (ROS) has an important role in the development and progression of leukemia. The application of pro-oxidant therapy, which targets redox dysregulation, has achieved satisfactory results in alleviating the conditions of and improving the survival rate for patients with ALL. However, drug resistance and side effects are two major challenges that must be addressed in pro-oxidant therapy. Oxidative stress can activate a variety of antioxidant mechanisms to help leukemia cells escape the damage caused by pro-oxidant drugs and develop drug resistance. Hematopoietic stem cells (HSCs) are extremely sensitive to oxidative stress due to their low levels of differentiation, and the use of pro-oxidant drugs inevitably causes damage to HSCs and may even cause severe bone marrow suppression.

acute lymphoblastic leukemia hematopoietic stem cells ROS oxidative stress pro-oxidative therapy

1. Introduction

Acute lymphoblastic leukemia (ALL) is a type of acute leukemia that mainly manifests as abnormal clonal proliferation of naive or mature T and B lymphocytes and their infiltration of bone marrow (BM), blood, or other organs and tissues, causing BM hematopoietic dysfunction and immune dysfunction. ALL has diverse biological characteristics and substantial clinical heterogeneity. With the continuous discovery of new therapeutic drugs and the ongoing innovation of treatment strategies, the remission rate and survival rate for patients with ALL continue to increase. However, the side effects of drugs and drug resistance in some patients remain problems requiring urgent resolution in clinical ALL treatment.

BM is a major hematopoietic organ. In a hypoxic BM niche, the balance between the production and clearance of reactive oxygen species (ROS) in normal hematopoietic stem cells (HSCs) is critical for the maintenance of normal physiological function. Sufficient evidence indicates that imbalances in redox homeostasis are involved in the development, progression, and relapse of leukemia [1]. Redox dysregulation and increased ROS production are well known and important characteristics of tumor cells, including leukemia cells [2]. In view of redox dysfunction, a weakness of leukemia cells, the implementation of pro-oxidant therapy has created hope for the elimination of leukemia cells. However, increased ROS levels can activate a variety of antioxidant mechanisms in leukemia cells to protect them from oxidative stress injury. In addition, leukemia cells can modify the BM niche into a leukemia

growth-permissive and normal hematopoiesis-suppressive leukemia niche, rendering the BM niche a sanctuary for leukemia cells to avoid damage from pro-oxidant agents [3][4][5]. These are important causes of leukemia cells' resistance to pro-oxidant drugs and ALL relapse.

During the implementation of pro-oxidant therapy for leukemia, the cytotoxic effect of ROS on cells is not selective. Pro-oxidant therapy is akin to a double-edged sword, affecting normal cells while killing tumor cells; for example, it has toxic effects on BM-derived HSCs and can induce side effects such as BM suppression, affecting the therapeutic effects on tumors and even endangering the lives of patients. Therefore, strengthening the cytotoxic effect of ROS on tumor cells while avoiding oxidative damage to normal HSCs has become an important issue in pro-oxidant therapy for leukemia.

2. ROS Sources and Effects

ROS are a class of oxygen metabolites that are more active than oxygen and oxygen-containing substances derived from them. The major species of ROS include superoxide, hydrogen peroxide (H_2O_2), and hydroxyl radicals [6]. ROS production in the body is the result of the aerobic metabolism of cells. Approximately 2% of the oxygen consumed by aerobic cells is estimated to be diverted to ROS generation [7].

Based on the source, ROS production can be divided into exogenous and endogenous production. Exogenous ROS are formed via stimulation by exogenous factors, such as radiation and drugs. Endogenous ROS are produced by mitochondrial and NADPH oxidase (NOX) pathways [8]. Mitochondria are the main site of ROS production in most eukaryotic cells. During aerobic respiration, most electrons are transported along the respiratory chain and combine with molecular oxygen to form water. However, a small portion of electrons leak out of respiratory chain enzyme complexes I and III, resulting in single-electron reduction in molecular oxygen to form strongly oxidative superoxide anions, which generate hydroxyl radicals and H_2O_2 through specific chemical reactions [9]. NOX, which is localized on the cell membrane, is a major source of ROS [10]. External signals, such as bacterial lipopolysaccharide, tumor necrosis factor-α (TNF-α), and interleukin (IL)-1, can stimulate rapid activation of NOX, resulting in a substantial increase in oxygen consumption by cells and a reduction in oxygen molecules to superoxide anions. Superoxide anions are catalyzed by dismutase to generate H_2O_2, leading to a rapid increase in ROS levels to eliminate invading pathogenic microorganisms. In addition, the endoplasmic reticulum (ER) and some enzymes, such as lipoxygenase, cyclooxygenase, and xanthine oxidase, can also generate ROS through chemical reactions [11].

Organisms have a complete antioxidant system, which is divided into enzymatic and nonenzymatic antioxidant systems. Enzymatic antioxidant systems include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). The main function of these antioxidant enzymes is to catalyze the degradation of peroxides. Among them, SOD and CAT are important components of the intracellular antioxidant defense system. SOD includes copper (Cu)/zinc (Zn)-SOD in the cytoplasm and nucleus and manganese (Mn)-SOD in mitochondria. SOD can catalyze superoxide anions to generate H_2O_2 and O_2. The main function of CAT in peroxisomes is to catalyze H_2O_2 to generate O_2 and H_2O [11]. GPx is an important intracellular enzyme, and in most cases, its activity

depends on the micronutrient cofactor selenium. Thus, GPx, which is often referred to as a selenocysteine peroxidase, can decompose H_2O_2 into O_2 and H_2O. GPx has a critical role in inhibiting lipid peroxidation and therefore protects cells from oxidative stress [12]. Nonenzymatic antioxidants include glutathione (GSH), thioredoxin (Trx), vitamin C, vitamin E, carotenoids, flavonoids, melatonin, and other compounds [13]. These nonenzymatic antioxidants either scavenge free radicals by acting as hydrogen donors to provide hydrogen ions or alleviate oxidative stress by chelating metal ions, trapping free radicals, and neutralizing peroxyl radicals [14][15]. Enzymatic and nonenzymatic antioxidant systems act synergistically to provide comprehensive antioxidant protection for cells and tissues.

Under normal circumstances, intracellular ROS production and clearance remain in dynamic equilibrium. An appropriate ROS level is necessary for the maintenance of normal physiological functions. ROS can regulate cell survival, growth, and differentiation and participate in immune and inflammatory responses. Ultraviolet radiation, pathogen invasion, and inflammation may disrupt the redox state of cells and affect the expression of specific genes through related signaling pathways, resulting in different physiological effects. Redox dysregulation, which is caused by various factors, can lead to overproduction and accumulation of ROS, causing oxidative damage to organelles, proteins, lipids, and DNA and thereby destroying the structural and functional integrity of cells and even causing cell death [16][17].

3. The Role of Redox Dyshomeostasis in the Occurrence of ALL

ALL is a highly heterogeneous hematopoietic malignancy whose etiology and pathogenesis are extremely complex and have yet to be fully elucidated. The occurrence of ALL is currently believed to not be caused by a single factor but may be the result of interactions among various factors, including genetics, infection, ionizing radiation, chemical substances, and immune dysfunction, in a complex environment. Clinical data indicate that most patients with ALL harbor acquired genetic alterations that contribute to the increased proliferation, prolonged survival, or impaired differentiation of lymphoid hematopoietic progenitors. Therefore, ALL can be regarded as a type of genetic disease [18].

To date, among the more than 200 reported chromosomal abnormalities in leukemias, balanced translocations are the most common, leading to the generation of fusion genes [19][20]. Abnormal gene expression due to chromosomal translocation or altered function of the encoded fusion protein impairs normal differentiation and yields an aberrant self-renewal capacity, thus having an important role in the malignant transformation of normal hematopoietic stem and progenitor cells (HSPCs). Translocation ETS leukemia-acute myeloid leukemia 1 (*ETV6-RUNX1*) is a chimeric transcription factor that is more common in childhood ALL. The incidence of *ETV6-RUNX1* is considerably higher than that of ALL, suggesting the occurrence of additional genetic events during leukemic transformation after birth [21]. Through the establishment of an *ETV6-RUNX1* transgenic mouse model, Kantner et al. found that although no notable hematopoietic abnormalities were observed in mice, the ROS level in B cells increased, and DNA damage also increased. These results indicated that expression of the oncogene *ETV6-RUNX1* might trigger the second strike by enhancing ROS production, eventually inducing leukemic transformation

[21]. Breakpoint cluster region-Abelson (BCR/ABL) is the most common chromosomal genetic abnormality in adult patients with ALL, with a positivity rate of 20–40% [22], and encodes proteins with tyrosine kinase activity, promotes cell proliferation, and inhibits apoptosis [23]. However, the incidence of BCR/ABL-positive samples far exceeds that of the associated leukemias, suggesting that the presence of the BCR/ABL fusion gene alone is insufficient to trigger leukemia [24]. Several recent studies have found that BCR-ABL oncoprotein-expressing cells are associated with a relative increase in intracellular ROS [25]. Studies have shown that BCR/ABL can influence ROS production through manipulation of the NOX complex [26]. In addition, BCR-ABL can also activate the PI3K/AKT/mTOR pathway to promote intracellular ROS production [25]. Compared with normal cells, BCR/ABL-positive cells suffer more oxidative DNA damage (including DNA double-strand breaks) and demonstrate an increased ability to survive DNA damage. BCR/ABL stimulates the efficiency but decreases the fidelity of the repair mechanisms of double-strand breaks, which may be the leading cause of the accumulation of chromosomal aberrations and subsequent malignant lesions [27].

To elucidate the role of ROS in the secondary gene events of ALL, Lim et al. recently investigated factors causing mutations in Janus kinase JAK3, JAK1, and Ikzf3 (encoding Aiolos) using a B-cell acute lymphoblastic leukemia (B-ALL) mouse model [28]. JAKs are nonreceptor tyrosine kinases. Activation of the JAK/signal transducer and activator of transcription (STAT) signaling pathway induces the transcription of genes involved in HSC differentiation and proliferation. [29]. Aiolos belongs to the Ikaros family and is an essential transcription factor for lymphocyte differentiation [30]. Abnormal expression of JAKs and Aiolos has been confirmed to be closely related to the development of ALL [29][31]. Lim et al. found that most mutations with low variant-allele frequency were associated with ROS-induced DNA damage. Application of the JAK inhibitor ruxolitinib can delay leukemia onset, reduce ROS and ROS-induced gene expression signatures, and alter ROS-induced mutational signatures, indicating that JAK mutations can alter the course of leukemia clonal evolution through ROS-induced DNA damage [28]. The clinical biochemical indices of patients with ALL showed a marked increase in the levels of malondialdehyde, an important biochemical index for plasma oxidative stress [32], and a notable increase in the levels of 8-oxodG and 8-OHdG, biomarkers of oxidative DNA damage in urine, in patients with ALL [33][34]. In addition, the level of oxidatively modified DNA bases in lymphocytes from children with ALL was markedly higher than that in children without the disease [35][36][37]. Abundant evidence has shown that ROS have a vital role in secondary gene events in ALL. However, the specific mechanism underlying the development and progression of ALL remains to be further elucidated.

According to the second hit theory, the occurrence of leukemia is the result of the accumulation of multiple gene abnormalities. Abnormal gene expression activates specific signaling pathways to promote the malignant transformation of cells. PI3K/AKT/mTOR, MAPK kinase (MEK)/extracellular signal-regulated kinase (ERK), and JAK/STAT are the major signaling pathways of oxidative stress and are also the representative signaling pathways for abnormal activation of leukemia cells [38][39]. In normal HSCs, overactivation of the above oxidative stress pathways promotes the production and intracellular accumulation of ROS, severely disturbs the normal biological functions of HSCs, and has an important role in leukemia progression [38][39]. Recent studies have shown that abnormal expression of a variety of genes activates the related oxidative stress pathway, thereby affecting ALL progression. These genes mainly include Notch, PTEN, RAS, and IL-7 and its receptor (IL-7R). (1) Notch encodes

a transmembrane receptor that regulates normal T cell development. Mutations in this gene are common in T cell acute lymphoblastic leukemia (T-ALL). In T-ALL cells, the withdrawal of Notch signals prevents stimulation of the mTOR pathway by mitogenic factors, indicating that Notch has a positive regulatory role in the mTOR pathway [40]. Mutant Notch can activate the mTOR pathway through the PI3K/AKT pathway or through activation of c-Myc without relying on the PI3K pathway [40][41]. (2) PTEN is a tumor suppressor gene closely related to tumor development. Its encoded protein has dual protein/lipid phosphatase activity and is a major phosphatase with a negative regulatory effect on the PI3K/AKT pathway [42]. Mutation or deactivation of PTEN after translation can result in chronic activation of PI3K/AKT/mTOR signaling in ALL cells, γ-secretase inhibitor (GSI) resistance, and inhibition of p53-mediated apoptosis [41]. (3) RAS is a proto-oncogene, and its encoded proteins are small GTPases, which act as molecular switches. Activated RAS promotes ROS production through NOX stimulation [43] and transduces signals from a variety of cell surface receptors to downstream signaling pathways, such as PI3K/AKT/mTOR and MAPK, to regulate a number of cell fate decisions. RAS-activating mutations and Notch mutations synergistically promote the development and progression of T-ALL [44]. (4) IL-7 and IL-7R are required for normal lymphocyte development. Without them, severe combined immunodeficiency occurs. However, excessive activation of IL-7/IL-7R signaling activates the three oxidative stress signaling pathways, PI3K/AKT/mTOR, MEK/ERK, and JAK/STAT, to promote the development of ALL and the resistance of ALL cells to chemotherapeutic drugs [28][45]. Lim et al. found that ROS induced by IL-7 in the downstream JAK/STAT pathway positively increased JAK/STAT signaling and that the occurrence of B-ALL and the survival of B-ALL cells were dependent on high levels of ROS driven by IL-7-dependent JAK/STAT signaling [28]. Silva et al. reported a positive feedback interaction between the IL-7-mediated PI3K/AKT signaling pathway and ROS. Moreover, activation of this pathway upregulated glucose transport protein type 1 and increased glucose uptake by T-ALL cells. The use of ROS scavengers inhibited the viability of T-ALL cells or even led to cell death [46]. These results suggest that ROS not only drive the development of ALL but are also indispensable for the survival of ALL cells. The major redox signaling pathways associated with ALL transformation are shown in Figure 1, and the corresponding targeted drugs are shown in Table 1.

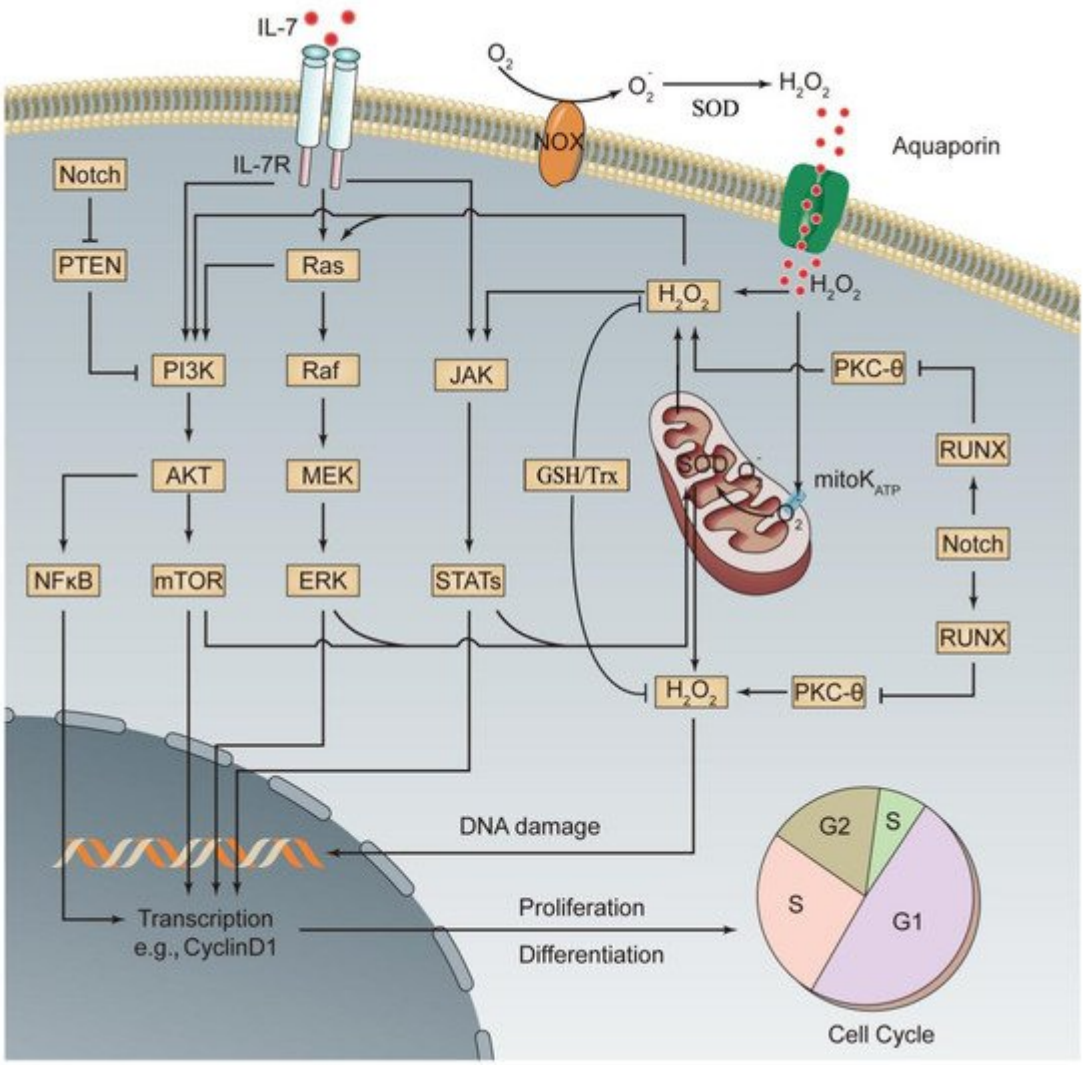

Figure 1. Activated oxidative stress signaling pathways involved in the pathogenesis of ALL. Increases in oncogene-, chemical drug-, or radiation-induced ROS production or abnormal expression of relevant genes leads to activation of three major oxidative stress signaling pathways, PI3K, MEK, and JAK, thus promoting the differentiation and proliferation of leukemia cells. Additionally, activation of oxidative stress signaling pathways promotes mitochondrial ROS production through enhanced oxidative metabolism, which further activates the three oxidative stress signaling pathways, thereby forming a positive feedback signaling pathway. A series of mechanisms in leukemia cells prevent excessive ROS production, thus avoiding cell injury or death (see the text for details). This figure was drawn based on existing research data; its accuracy and more precise signaling mechanisms must be confirmed and supplemented by extensive, in-depth studies. mitoK$_{ATP}$, mitochondrial ATP-sensitive K^+ channel.

Table 1. ALL redox signaling pathway targets and representative drugs.

Signaling Pathway	Targets	Representative Drugs	Antileukemic Effect	Refs
BCR/ABL	Tyrosine kinase inhibitor	Imatinib	First-generation TKI that can block the ATP-binding sites of BCR-ABL and prevent	[47]

Signaling Pathway	Targets	Representative Drugs	Antileukemic Effect	Refs
	(TKI)		activation of the conformation of oncogenic proteins	
		Nilotinib	Second-generation TKI and high-affinity aminopyrimidine-based ATP-competitive inhibitor with more specific inhibition of BCR/ABL activity	[48]
		Dasatinib	Second-generation TKI that can bind to inactive and active BCR/ABL kinase and inhibit Src family kinases and c-Kit	[48]
		Bosutinib	Third-generation TKI and potent dual inhibitor of Src and ABL kinases with longer-term safety than second-generation and other third-generation TKIs	[49]
		Ponatinib	Third-generation TKI that is effective for known mutations in imatinib-resistant genes (including T315I)	[50]
Notch	γ-secretase inhibitor (GSIs)	BMS-906024	Inhibits the activity of Notch signaling by downregulating the expression of multiple known target genes of Notch but has no marked effect on c-Myc	[51]
		PF-03084014	Downregulates the level of the Notch intracellular domain and the expression of Notch target genes Hes-1 and c-Myc and induces cell cycle arrest and apoptosis of T-ALL cells	[52]
PI3K/AKT/mTOR	PI3K-δ inhibitor	Idelalisib	Downregulates the level of AKT phosphorylation in B-ALL cells, inhibits cell proliferation, and blocks the homing of B-ALL cells into the bone marrow	[53]
		NVP-BKM120	Downregulates the phosphorylation levels of AKT and mTOR in T-ALL cells, inhibits cell cycle progression, and promotes apoptosis	[54]
	AKT inhibitor	MK-2206	Downregulates AKT phosphorylation levels in both T-ALL and B-ALL cell lines (it can also promote PTEN phosphorylation in B-ALL cell lines), inhibits cell proliferation, and promotes apoptosis	[55]
	PI3K/mTOR inhibitor	PI-103	More potent than inhibitors that are selective only for PI3K or for mTOR and can effectively	[56]

Signaling Pathway	Targets	Representative Drugs	Antileukemic Effect	Refs
			induce cell cycle arrest and apoptosis in T-ALL cells	
JAK/STAT	JAK inhibitor	Ruxolitinib	JAK1/2 inhibitor that can reduce ROS and ROS-induced gene expression signatures and inhibit the growth of leukemia cells	[28]
RAS	MEK inhibitor	Selumetinib Trametinib MEK162	Reduce ERK phosphorylation and induce apoptosis in the RAS-mutant MLL-rearranged ALL cells	[57]

References

Exp. Clin. Cancer Res. 2018, 37, 125.

3. Burt, R.; Dey, A.; Aref, S.; Aguiar, M.; Akarca, A.; Bailey, K.; Day, W.; Hooper, S.; Kirkwood, A.; Kirschner, K.; et al. Activated stromal cells transfer mitochondria to rescue acute lymphoblastic leukemia cells from oxidative stress. Blood 2019, 134, 1415–1429.
4. Polak, R.; de Rooij, B.; Pieters, R.; den Boer, M.L. B-cell precursor acute lymphoblastic leukemia cells use tunneling nanotubes to orchestrate their microenvironment. Blood 2015, 126, 2404–2414.
5. Liu, J.; Masurekar, A.; Johnson, S.; Chakraborty, S.; Griffiths, J.; Smith, D.; Alexander, S.; Dempsey, C.; Parker, C.; Harrison, S.; et al. Stromal cell-mediated mitochondrial redox adaptation regulates drug resistance in childhood acute lymphoblastic leukemia. Oncotarget 2015, 6, 43048–43064.
6. Chen, Y.F.; Liu, H.; Luo, X.J.; Zhao, Z.; Zou, Z.Y.; Li, J.; Lin, X.J.; Liang, Y. The roles of reactive oxygen species (ROS) and autophagy in the survival and death of leukemia cells. Crit. Rev. Oncol. Hematol. 2017, 112, 21–30.
7. Sohal, R.S.; Weindruch, R. Oxidative stress, caloric restriction, and aging. Science 1996, 273, 59–63.
8. Zou, Z.; Chang, H.; Li, H.; Wang, S. Induction of reactive oxygen species: An emerging approach for cancer therapy. Apoptosis 2017, 22, 1321–1335.
9. Cao, H.; Wang, Y.; Wang, Q.; Wang, R.; Guo, S.; Zhao, X.; Zhang, Y.; Tong, D.; Yang, Z. Taxol prevents myocardial ischemia-reperfusion injury by inducing JNK-mediated HO-1 expression. Pharm. Biol. 2016, 54, 555–560.
10. Leto, T.L.; Morand, S.; Hurt, D.; Ueyama, T. Targeting and regulation of reactive oxygen species generation by Nox family NADPH oxidases. Antioxid. Redox Signal. 2009, 11, 2607–2619.
11. Chen, Y.; Luo, X.; Zou, Z.; Liang, Y. The Role of Reactive Oxygen Species in Tumor Treatment and its Impact on Bone Marrow Hematopoiesis. Curr. Drug Targets 2020, 21, 477–498.

4. Redox Regulation in Leukemia

The hypoxic BM niche is not only a habitat for HSCs but also a natural sanctuary for malignant blood cells. Leukemia cells hidden in the BM niche can avoid the cytotoxicity of chemotherapeutic drugs, resulting in the formation of minimal residual disease. Minimal residual disease is an accepted root cause of drug resistance and relapse in leukemia [58]. A large body of evidence has shown that in a hypoxic environment, the ROS level and its regulation have important roles in leukemia cell survival, proliferation, differentiation, immune escape, and epigenetic changes [59].

Similar to HSCs, primitive leukemia cells also have self-renewal, self-differentiation, and self-proliferation capacities, and their relatively low intracellular ROS levels are conducive to cell survival and stemness maintenance [60][61]. Compared with that in leukemia cells with higher proliferating activity, the ROS level in primitive leukemia cells is lower, this phenomenon has been observed in many types of leukemia cells [2][61]. Gianfelici et al. also observed that the most aggressive leukemia-initiating cells (LICs) in T-ALL model mice and human T-ALL were characterized by low levels of ROS, and that the increase in ROS levels inhibited the activity of LICs [62], suggesting that the behavior of ALL cells is closely related to ROS levels and oxidative stress status.

Similar to other types of cancer cells, leukemia cells can also undergo glycolysis for energy metabolism. However, a large amount of evidence indicates that leukemia cells mainly gain energy through mitochondrial respiration and that ROS produced through the mitochondrial respiratory chain thus become a major source of ROS in leukemia cells [63][26]. Markedly improved NOX activity has been observed in many leukemia cell lines, including AML, chronic myelogenous leukemia, and promyelocytic leukemia cell lines, indicating that constitutive activation of NOX is another important source of ROS in leukemia cells [64][65][66]. In the in vitro culture of T-ALL cells, the inhibition of complex I of the respiratory chain with rotenone and the disassembly of NADPH subunits with apocynin both abrogated the IL-7-mediated elevation of intracellular ROS levels, confirming that the mitochondrial respiratory chain and NOX are also the main sources of intracellular ROS production in T-ALL cells [46]. Studies have found that in lymphoblastic leukemia cells, xanthine dehydrogenase and xanthine oxidase can oxidize NADH to catalyze the production of ROS, suggesting that in addition to the mitochondrial respiratory chain and NOX complex, certain metabolic/detoxification pathways in lymphoblastic leukemia cells are also important sources of ROS production

12. Ighodaro, O.M.; Akinloye, O.A. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J. Med. 2018, 54, 287–293.

13. Zhu, R.; Wang, Y.; Zhang, L.; Guo, Q. Oxidative stress and liver disease. Hepatol. Res. 2012, 42, 741–749.

14. Burup, E.A.; Taylor, Y.C.; Brown, J.M. Role of glutathione in the hypoxic cytotoxicity of misonidazole. Cancer Res. 1983, 43, 997–1002.

15. Lu, J.; Holmgren, A. The thioredoxin antioxidant system. Free Radic. Biol. Med. 2014, 66, 75–87.

16. Franco, R.; Schoneveld, O.; Georgakilas, A.G.; Panayiotidis, M.I. Oxidative stress, DNA methylation and carcinogenesis. Cancer Lett. 2008, 266, 6–11.

17. Chen, Y.; Zou, Z.; Wu, Z.; Zhao, Z.; Luo, X.; Xie, C.; Liang, Y. TNF-α-induced programmed cell death in the pathogenesis of acquired aplastic anemia. Expert Rev. Hematol. 2015, 8, 515–526.

18. Mrózek, K.; Harper, D.P.; Aplan, P.D. Cytogenetics and molecular genetics of acute lymphoblastic leukemia. Hematol. Oncol. Clin. N. Am. 2009, 23, 991–1010.

19. Greaves, M.F. Biological models for leukaemia and lymphoma. IARC Sci. Publ. 2004, 157, 361–372.

20. Marincevic-Zuniga, Y.; Dahlberg, J.; Nilsson, S.; Raine, A.; Nystedt, S.; Lindqvist, C.M.; Berglund, E.C.; Abrahamsson, J.; Cavelier, L.; Forestier, E.; et al. Transcriptome sequencing in pediatric acute lymphoblastic leukemia identifies fusion genes associated with distinct DNA methylation profiles. J. Hematol. Oncol. 2017, 10, 148.

21. Kamper, P.; Ware, M.D.; Dolgu, A.; Bauer, E.; Esterbauer, H.; Casanova, E.; Sexl, V.; Stoiber, D. ETV6/RUNX1 induces reactive oxygen species and drives the accumulation of DNA damage in B cells. Neoplasia 2013, 15, 1292–1300.

22. Juric, D.; Lacayo, N.J.; Ramsey, M.C.; Racevskis, J.; Wiernik, P.H.; Rowe, J.M.; Goldstone, A.H.; O'Dwyer, P.J.; Paietta, E.; Sikic, B.I. Differential gene expression patterns and interaction networks in BCR-ABL-positive and -negative adult acute lymphoblastic leukemias. J. Clin. Oncol. 2007, 25, 1341–1349.

23. Parada, Y.; Banerji, L.; Glassford, J.; Lea, N.C.; Collado, M.; Rivas, C.; Lewis, J.L.; Gordon, M.Y.; Thomas, N.S.; Lam, E.W. BCR-ABL and interleukin 3 promote haematopoietic cell proliferation and survival through modulation of cyclin D2 and p27Kip1 expression. J. Biol. Chem. 2001, 276, 23572–23580.

24. Basecke, J.; Griesinger, F.; Trumper, L.; Brittinger, G. Leukemia- and lymphoma-associated genetic aberrations in healthy individuals. Ann. Hematol. 2002, 81, 64–75.

25. Kim, J.H.; Chu, S.C.; Gramlich, J.L.; Pride, Y.B.; Babendreier, E.; Chauhan, D.; Salgia, R.; Podar, K.; Griffin, J.D.; Sattler, M. Activation of the PI3K/mTOR pathway by BCR-ABL contributes to increased production of reactive oxygen species. Blood 2005, 105, 1717–1723.
26. Irwin, M.E.; Rivera-Del Valle, N.; Chandra, J. Redox control of leukemia: From molecular mechanisms to therapeutic opportunities. Antioxid. Redox Signal. 2013, 18, 1349–1383.
27. Kostyrko, K.; Cramer, K.; Stupfanek, A.; Richardson, C.; Skorski, T. BCR/ABL promotes accumulation of chromosomal aberrations induced by oxidative and genotoxic stress. Leukemia 2008, 22, 1969–1972.
28. Lim, M.; Batista, C.R.; de Oliveira, B.R.; Creighton, R.; Ferguson, J.; Clemmer, K.; Knight, D.; Iansavitchous, J.; Mahmood, D.; Avino, M.; et al. Janus Kinase Mutations in Mice Lacking PU.1 and Spi-B Drive B Cell Leukemia through Reactive Oxygen Species-Induced DNA Damage. Mol. Cell Biol. 2020, 40, e00189-20.
29. Steeghs, E.M.P.; Jerchel, I.S.; de Goffau-Nobel, W.; Hoogkamer, A.Q.; Boer, J.M.; Boeree, A.; van de Ven, C.; Koudijs, M.J.; Besselink, N.J.M.; de Groot-Kruseman, H.A.; et al. JAK2 aberrations in childhood B-cell precursor acute lymphoblastic leukemia. Oncotarget 2017, 8, 89923–89938.
30. Georgopoulos, K. The making of a lymphocyte: The choice among disparate cell fates and the IKAROS enigma. Genes Dev. 2017, 31, 439–450.
31. Holmfeldt, L.; Wei, L.; Diaz-Flores, E.; Walsh, M.; Zhang, J.; Ding, L.; Payne-Turner, D.; Churchman, M.; Andersson, A.; Chen, S.C.; et al. The genomic landscape of hypodiploid acute lymphoblastic leukemia. Nat. Genet. 2013, 45, 242–252.
32. Rasool, M.; Farooq, S.; Malik, A.; Shaukat, A.; Manan, A.; Asif, M.; Sani, S.; Qazi, M.H.; Kamal, M.A.; Iqbal, Z.; et al. Assessment of circulating biochemical markers and antioxidative status in acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) patients. Saudi. J. Biol. Sci. 2015, 22, 106–111.
33. Almondes, K.G.; de Oliveira, T.F.; Siviero-Miachon, A.A.; Lee, M.L.; Rondó, P.H.; Loureiro, A.P.; Spinola-Castro, A.M.; Cozzolino, S.M. Selenium inadequacy is not associated with oxidative stress in child and adolescent acute lymphocytic leukemia survivors. Nutrition 2014, 30, 563–568.
34. Yang, Y.; Tian, Y.; Yan, C.; Jin, X.; Tang, J.; Shen, X. Determinants of urinary 8-hydroxy-2′-deoxyguanosine in Chinese children with acute leukemia. Environ. Toxicol. 2009, 24, 446–452.
35. Dincer, Y.; Yüksel, S.; Batar, B.; Güven, M.; Onaran, I.; Celkan, T. DNA Repair Gene Polymorphisms and Their Relation with DNA Damage, DNA Repair, and Total Antioxidant Capacity in Childhood Acute Lymphoblastic Leukemia Survivors. J. Pediatr. Hematol. Oncol. 2015, 37, 344–350.
36. Sentürker, S.; Karahalil, B.; Inal, M.; Yilmaz, H.; Müslümanoglu, H.; Gedikoglu, G.; Dizdaroglu, M. Oxidative DNA base damage and antioxidant enzyme levels in childhood acute lymphoblastic

leukemia. FEBS Lett. 1997, 416, 286–290.

37. Olinski, R.; Styczynski, J.; Olinska, E.; Gackowski, D. Viral infection-oxidative stress/DNA damage-aberrant DNA methylation: Separate or interrelated events responsible for genetic instability and childhood ALL development? Biochim. Biophys. Acta 2014, 1846, 226–231.
38. Steelman, L.S.; Abrams, S.L.; Whelan, J.; Bertrand, F.E.; Ludwig, D.E.; Bäsecke, J.; Libra, M.; Stivala, F.; Milella, M.; Tafuri, A.; et al. Contributions of the Raf/MEK/ERK, PI3K/PTEN/Akt/mTOR and Jak/STAT pathways to leukemia. Leukemia 2008, 22, 686–707.
39. Chen, C.; Liu, Y.; Liu, Y.; Zheng, P. The axis of mTOR-mitochondria-ROS and stemness of the hematopoietic stem cells. Cell Cycle 2009, 8, 1158–1160.
40. Chan, S.M.; Weng, A.P.; Tibshirani, R.; Aster, J.C.; Utz, P.J. Notch signals positively regulate activity of the mTOR pathway in T-cell acute lymphoblastic leukemia. Blood 2007, 110, 278–286.
41. Hales, E.C.; Taub, J.W.; Matherly, L.H. New insights into Notch1 regulation of the PI3K-AKT-mTOR1 signaling axis: Targeted therapy of γ-secretase inhibitor resistant T-cell acute lymphoblastic leukemia. Cell Signal 2014, 26, 149–161.
42. Mocanu, M.M.; Yellon, D.M. PTEN, the Achilles' heel of myocardial ischaemia/reperfusion injury? Br. J. Pharmacol. 2007, 150, 833–838.
43. Hole, P.S.; Pearn, L.; Tonks, A.J.; James, P.E.; Burnett, A.K.; Darley, R.L.; Tonks, A. Ras-induced reactive oxygen species promote growth factor-independent proliferation in human CD34+ hematopoietic progenitor cells. Blood 2010, 115, 1238–1246.
44. Bongiovanni, D.; Saccomani, V.; Piovan, E. Aberrant Signaling Pathways in T-Cell Acute Lymphoblastic Leukemia. Int. J. Mol. Sci. 2017, 18, 1904.
45. Oliveira, M.L.; Akkapeddi, P.; Ribeiro, D.; Melão, A.; Barata, J.T. IL-7R-mediated signaling in T-cell acute lymphoblastic leukemia: An update. Adv. Biol. Regul. 2019, 71, 88–96.
46. Silva, A.; Gírio, A.; Cebola, I.; Santos, C.I.; Antunes, F.; Barata, J.T. Intracellular reactive oxygen species are essential for PI3K/Akt/mTOR-dependent IL-7-mediated viability of T-cell acute lymphoblastic leukemia cells. Leukemia 2011, 25, 960–967.
47. Lee, H.J.; Thompson, J.E.; Wang, E.S.; Wetzler, M. Philadelphia chromosome-positive acute lymphoblastic leukemia: Current treatment and future perspectives. Cancer 2011, 117, 1583–1594.
48. Piccaluga, P.P.; Paolini, S.; Martinelli, G. Tyrosine kinase inhibitors for the treatment of Philadelphia chromosome-positive adult acute lymphoblastic leukemia. Cancer 2007, 110, 1178–1186.
49. Varallo-Rodriguez, C.; Freyer, C.W., Jr.; Ontiveros, E.P.; Griffiths, E.A.; Wang, E.S.; Wetzler, M. Bosutinib for the Treatment of Philadelphia Chromosome-Positive Leukemias. Expert Opin.

Orphan Drugs 2015, 3, 599–608.

50. Sanford, D.S.; Kantarjian, H.; O'Brien, S.; Jabbour, E.; Cortes, J.; Ravandi, F. The role of ponatinib in Philadelphia chromosome-positive acute lymphoblastic leukemia. Expert Rev. Anticancer Ther. 2015, 15, 365–373.
51. Knoechel, B.; Bhatt, A.; Pan, L.; Pedamallu, C.S.; Severson, E.; Gutierrez, A.; Dorfman, D.M.; Kuo, F.C.; Kluk, M.; Kung, A.L.; et al. Complete hematologic response of early T-cell progenitor acute lymphoblastic leukemia to the γ-secretase inhibitor BMS-906024: Genetic and epigenetic findings in an outlier case. Cold Spring Harb. Mol. Case Stud. 2015, 1, a000539.
52. Wei, P.; Walls, M.; Qiu, M.; Ding, R.; Denlinger, R.H.; Wong, A.; Tsaparikos, K.; Jani, J.P.; Hosea, N.; Sands, M.; et al. Evaluation of selective gamma-secretase inhibitor PF-03084014 for its antitumor efficacy and gastrointestinal safety to guide optimal clinical trial design. Mol. Cancer Ther. 2010, 9, 1618–1628.
53. Rosin, N.Y.; Koehrer, S.; Kim, E.; O'Brien, S.; Wierda, W.G.; Thomas, D.A.; Estrov, Z.; Kantarjian, H.M.; Lannutti, B.J.; Burger, J.A. In Vitro Effects of PI3Kδ Inhibitor GS-1101 (Cal-101) in Acute Lymphoblastic Leukemia (ALL). Blood 2012, 120, 3534.
54. Pereira, J.K.; Machado-Neto, J.A.; Lopes, M.R.; Morini, B.C.; Traina, F.; Costa, F.F.; Saad, S.T.; Favaro, P. Molecular effects of the phosphatidylinositol-3-kinase inhibitor NVP-BKM120 on T and B-cell acute lymphoblastic leukaemia. Eur. J. Cancer 2015, 51, 2076–2085.
55. Naderali, E.; Valipour, B.; Khaki, A.A.; Soleymani Rad, J.; Alihemmati, A.; Rahmati, M.; Nozad Charoudeh, H. Positive Effects of PI3K/Akt Signaling Inhibition on PTEN and P53 in Prevention of Acute Lymphoblastic Leukemia Tumor Cells. Adv. Pharm. Bull. 2019, 9, 470–480.
56. Chiarini, F.; Falà, F.; Tazzari, P.L.; Ricci, F.; Astolfi, A.; Pession, A.; Pagliaro, P.; McCubrey, J.A.; Martelli, A.M. Dual inhibition of class IA phosphatidylinositol 3-kinase and mammalian target of rapamycin as a new therapeutic option for T-cell acute lymphoblastic leukemia. Cancer Res. 2009, 69, 3520–3528.
57. Kerstjens, M.; Driessen, E.M.; Willekes, M.; Pinhanços, S.S.; Schneider, P.; Pieters, R.; Stam, R.W. MEK inhibition is a promising therapeutic strategy for MLL-rearranged infant acute lymphoblastic leukemia patients carrying RAS mutations. Oncotarget 2017, 8, 14835–14846.
58. Moses, B.S.; Slone, W.L.; Thomas, P.; Evans, R.; Piktel, D.; Angel, P.M.; Walsh, C.M.; Cantrell, P.S.; Rellick, S.L.; Martin, K.H.; et al. Bone marrow microenvironment modulation of acute lymphoblastic leukemia phenotype. Exp. Hematol. 2016, 44, 50–59.e1-2.
59. Zhou, F.; Shen, Q.; Claret, F.X. Novel roles of reactive oxygen species in the pathogenesis of acute myeloid leukemia. J. Leukoc. Biol. 2013, 94, 423–429.
60. Herault, O.; Hope, K.J.; Deneault, E.; Mayotte, N.; Chagraoui, J.; Wilhelm, B.T.; Cellot, S.; Sauvageau, M.; Andrade-Navarro, M.A.; Hébert, J.; et al. A role for GPx3 in activity of normal and

leukemia stem cells. J. Exp. Med. 2012, 209, 895–901.

61. Lagadinou, E.D.; Sach, A.; Callahan, K.; Rossi, R.M.; Neering, S.J.; Minhajuddin, M.; Ashton, J.M.; Pei, S.; Grose, V.; O'Dwyer, K.M.; et al. BCL-2 inhibition targets oxidative phosphorylation and selectively eradicates quiescent human leukemia stem cells. Cell Stem Cell 2013, 12, 329–341.
62. Giambra, V.; Jenkins, C.R.; Wang, H.; Lam, S.H.; Shevchuk, O.O.; Nemirovsky, O.; Wai, C.; Gusscott, S.; Chiang, M.Y.; Aster, J.C.; et al. NOTCH1 promotes T cell leukemia-initiating activity by RUNX-mediated regulation of PKC-θ and reactive oxygen species. Nat. Med. 2012, 18, 1693–1698.
63. Filippi, M.D.; Ghaffari, S. Mitochondria in the maintenance of hematopoietic stem cells: New perspectives and opportunities. Blood 2019, 133, 1943–1952.
64. Hole, P.S.; Zabkiewicz, J.; Munje, C.; Newton, Z.; Pearn, L.; White, P.; Marquez, N.; Hills, R.K.; Burnett, A.K.; Tonks, A.; et al. Overproduction of NOX-derived ROS in AML promotes proliferation and is associated with defective oxidative stress signaling. Blood 2013, 122, 3322–3330.
65. Singh, M.M.; Irwin, M.E.; Gao, Y.; Ban, K.; Shi, P.; Arlinghaus, R.B.; Amin, H.M.; Chandra, J. Inhibition of the NADPH oxidase regulates heme oxygenase 1 expression in chronic myeloid leukemia. Cancer 2012, 118, 3433–3445.
66. Dong, J.M.; Zhao, S.G.; Huang, G.Y.; Liu, Q. NADPH oxidase-mediated generation of reactive oxygen species is critically required for survival of undifferentiated human promyelocytic leukemia cell line HL-60. Free Radic. Res. 2004, 38, 629–637.
67. Zhang, Z.; Blake, D.R.; Stevens, C.R.; Kanczler, J.M.; Winyard, P.G.; Symons, M.C.; Benboubetra, M.; Harrison, R. A reappraisal of xanthine dehydrogenase and oxidase in hypoxic reperfusion injury: The role of NADH as an electron donor. Free Radic. Res. 1998, 28, 151–164.
68. Battisti, V.; Maders, L.D.; Bagatini, M.D.; Santos, K.F.; Spanevello, R.M.; Maldonado, P.A.; Brulé, A.O.; Araújo Mdo, C.; Schetinger, M.R.; Morsch, V.M. Measurement of oxidative stress and antioxidant status in acute lymphoblastic leukemia patients. Clin. Biochem. 2008, 41, 511–518.
69. Gaman, A.M.; Buga, A.M.; Gaman, M.A.; Popa-Wagner, A. The role of oxidative stress and the effects of antioxidants on the incidence of infectious complications of chronic lymphocytic leukemia. Oxid. Med. Cell Longev. 2014, 2014, 158135.
70. Tahir, I.M.; Iqbal, T.; Jamil, A.; Saqib, M. Association of BCL-2 with oxidative stress and total antioxidant status in pediatric acute lymphoblastic leukemia. J. Biol. Regul. Homeost. Agents 2017, 31, 1023–1027.
71. Ben Mahmoud, L.; Mdhaffar, M.; Ghozzi, H.; Ammar, M.; Hakim, A.; Atheymen, R.; Sahnoun, Z.; Elloumi, M.; Zeghal, K. Oxidative Stress in Tunisian Patients with Acute Lymphoblastic Leukemia and Its Involvement in Leukemic Relapse. J. Pediatr. Hematol. Oncol. 2017, 39, e124–e130.

72. Nishiura, T.; Suzuki, K.; Kawaguchi, T.; Nakao, H.; Kawamura, N.; Taniguchi, M.; Kanayama, Y.; Yonezawa, T.; Iizuka, S.; Taniguchi, N. Elevated serum manganese superoxide dismutase in acute leukemias. Cancer Lett. 1992, 62, 211–215.
73. Schoeneberger, H.; Belz, K.; Schenk, B.; Fulda, S. Impairment of antioxidant defense via glutathione depletion sensitizes acute lymphoblastic leukemia cells for Smac mimetic-induced cell death. Oncogene 2015, 34, 4032–4043.
74. Silic-Benussi, M.; Scattolin, G.; Cavallari, I.; Minuzzo, S.; Del Bianco, P.; Francescato, S.; Basso, G.; Indraccolo, S.; D'Agostino, D.M.; Ciminale, V. Selective killing of human T-ALL cells: An integrated approach targeting redox homeostasis and the OMA1/OPA1 axis. Cell Death Dis. 2018, 9, 822.
75. Jasek-Gajda, E.; Jurkowska, H.; Jasińska, M.; Lis, G.J. Targeting the MAPK/ERK and PI3K/AKT Signaling Pathways Affects NRF2, Trx and GSH Antioxidant Systems in Leukemia Cells. Antioxidants 2020, 9, 633.
76. Chen, Y.; Liang, Y.; Luo, X.; Hu, Q. Oxidative resistance of leukemic stem cells and oxidative damage to hematopoietic stem cells under pro-oxidative therapy. Cell Death Dis. 2020, 11, 291.
77. Rustom, A.; Saffrich, R.; Markovic, I.; Walther, P.; Gerdes, H.H. Nanotubular highways for intercellular organelle transport. Science 2004, 303, 1007–1010.