Reactive Oxygen Species in Acute Lymphoblastic Leukaemia

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Acute lymphoblastic leukaemia (ALL) is the most common cancer diagnosed in children and adolescents. Approximately 70% of patients survive >5-years following diagnosis, however, for those that fail upfront therapies, survival is poor. Reactive oxygen species (ROS) are elevated in a range of cancers and are emerging as significant contributors to the leukaemogenesis of ALL. ROS modulate the function of signalling proteins through oxidation of cysteine residues, as well as promote genomic instability by damaging DNA, to promote chemotherapy resistance. Current therapeutic approaches exploit the pro-oxidant intracellular environment of malignant B and T lymphoblasts to cause irreversible DNA damage and cell death, however these strategies impact normal haematopoiesis and lead to long lasting side-effects. Therapies suppressing ROS production, especially those targeting ROS producing enzymes such as the NADPH oxidases (NOXs), are emerging alternatives to treat cancers and may be exploited to improve the ALL treatment.

acute lymphoblastic leukaemia | reactive oxygen species | oxidative stress | NADPH oxidases | antioxidants | redox homeostasis | second messenger signalling | oxidative DNA damage | resistance | oncogenic signalling | cysteine oxidation | kinase | phosphatase

1. Introduction

Acute lymphoblastic leukaemia (ALL) is a heterogeneous malignancy of immature B or T lymphoblasts, which rapidly proliferate in the bone marrow, blood, and some extra-medullary sites such as the spleen and lymph nodes [1]. ALL is the most common form of childhood cancer [2], and although only 20% of ALL diagnoses are diagnosed in adults, four out of five deaths from ALL are in this age group [3]. The overall survival (OS) of children diagnosed with ALL has dramatically improved over the last 40 years. Indeed, the development of multidrug treatment regimens including vincristine [1][4], corticosteroids [5], and asparaginase [6], with most regimens adding an anthracycline [7] (usually doxorubicin or daunorubicin) has reduced treatment resistance, and led to remission rates of greater than 80% [4]. The backbone of ALL treatment is similar in adults; however, they have worse outcomes due to both higher risk disease features at diagnosis and more toxicities associated with therapies [1][4][8]. However, in both populations the early failure of upfront therapies has devastating consequences, with a median 5-year OS of 21% for children [8] and 2% for adults who relapse within their first year of diagnosis [9]. These concerning data highlight the need to continually develop treatments for ALL patients at diagnosis and at disease progression.

A handful of risk factors are associated with ALL including, prenatal exposure to X-rays, postnatal exposure to high doses of radiation and previous treatments with chemotherapy [10], with a genetic predisposition seen in a subset of ALL cases. These include rare genetic and familial cancer syndromes, DNA polymorphisms in non-coding genes and numerous germline variants in coding genes [11]. Chromosomal abnormalities are also common in ALL and include gain or loss of chromosomal content (aneuploidy) and chromosomal rearrangements. Typical chromosomal translocations include, t(9;22) [BCR/ABL1], t(12;21) [ETV6/RUNX1], t(1;19) [TCF3/PBX1], and Mixed-lineage leukaemia (MLL)-rearrangements [1]. Overall consequences of chromosomal abnormalities are the loss of tumour suppressor genes or production of chimeric proteins that dysregulate many cellular processes particularly those that underpin cellular development, differentiation, multiplications, and cell cycle regulation [4]. Recurring somatic and occasionally germline mutations in transcription factors (*IKZF1, STAT5*) [12][13], tumour suppressors *TP53* (including germline variants), *CDKN2A* [14][15], and signalling pathways genes such as NOTCH1 [16], PI3K/Akt (*FLT3, PTEN, PTPN11*) [17][18][19][20], JAK/STAT (*CLRF2, IL7R, JAK1, JAK3*) [21][22] and Ras (*BCR/ABL, NRAS, KRAS*) [23][24] drive malignant transformation of immature lymphocytes and perturb the function of the body's immune system. Recurring mutations in signalling genes are strongly associated with pathways that underpin the increased production of reactive oxygen species (ROS); oxidative radicals that induce DNA damage leading to genomic instability and promote leukaemogenesis [25]. However, the roles of ROS in redox signalling and genome instability in ALL remains enigmatic and infantine.

2. Redox Dysregulation in ALL

Multiple lines of evidence support the notion that ROS play a role in the leukaemogenesis of ALL. ALL associated somatic heterogeneity and chromosomal translocations trigger constitutive activation of various oncogenic kinases, well-established contributors to increased ROS production in leukaemia [25]. Multiple pathways/mechanisms are involved in the generation of increased ROS in ALL (**Figure 1**). Some of the better characterised of these proteins/pathways are discussed below.

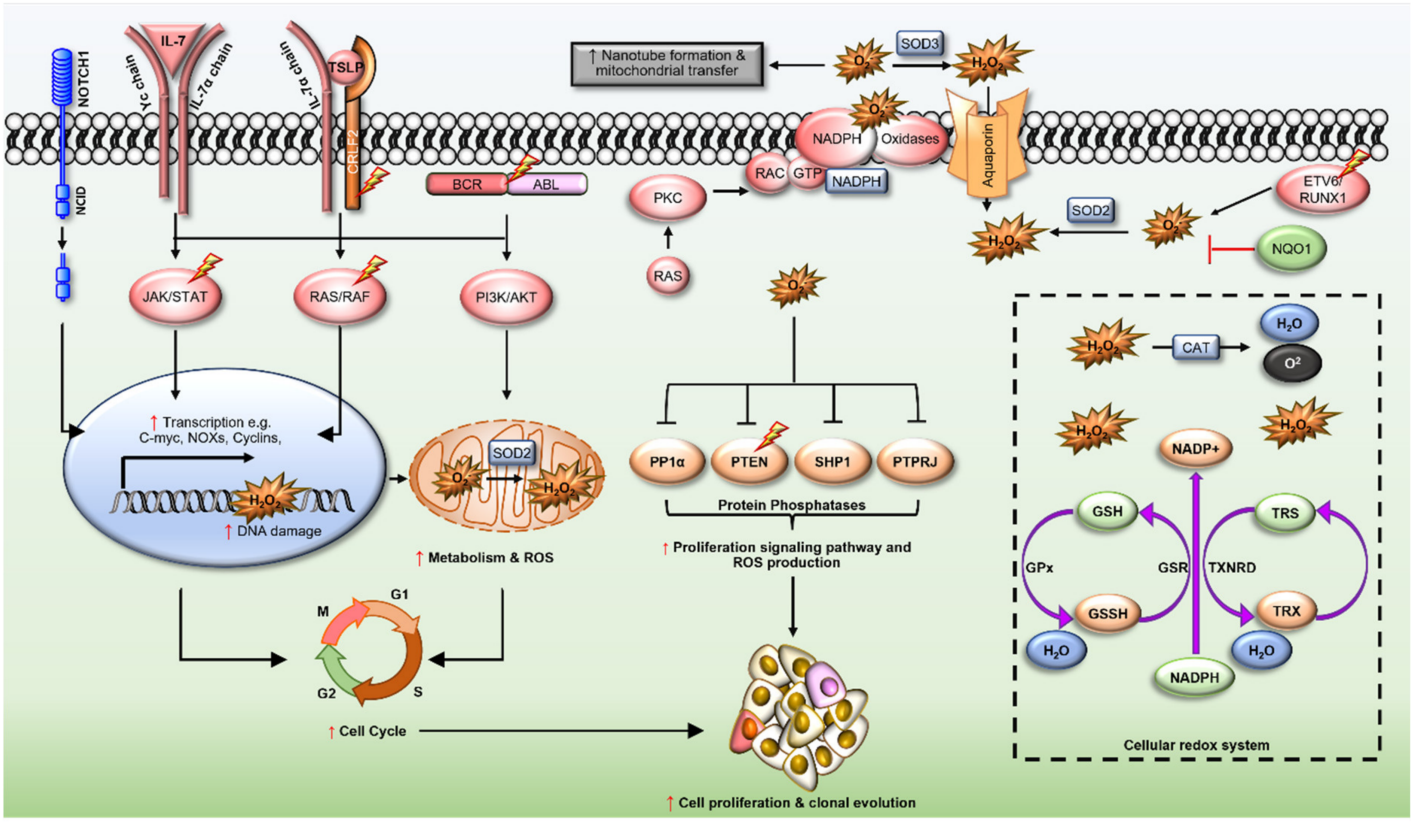

Figure 1. Signalling pathways linked to reactive oxygen species production in acute lymphoblastic leukaemia. Recurring somatic mutations to *CRLF2*, *JAK*, *NQO1*, *NOTCH1* and *RAS*, chromosomal translocations such as BCR/ABL and ETV6/RUNX1 as well as overexpression of CRLF2 and IL7 receptor drive excessive production of intracellular reactive oxygen species (ROS) production (superoxide- $O_2^{\cdot -}$ and hydrogen peroxide- H_2O_2) in acute lymphoblastic leukaemia (ALL). High-level ROS production drives redox signalling through oxidative posttranslational modifications that increase the activity of kinases and inactivate protein tyrosine phosphatases, and cause lipid peroxidation and genomic instability leading to leukaemia progression and chemotherapy resistance. Cellular redox systems (shown in the hashed rectangle) regulate ROS homeostasis by converting H_2O_2 to water and suppress ROS induced apoptosis. Red shapes = proteins with increased activity or expression; dark green = proteins with decrease activity or expression; orange = oxidised proteins; light green = reduced proteins.

2.1. ETV6/RUNX1 Fusions

Hallmark ALL associated chromosomal abnormalities drive malignant transformation of HSCs and progenitor cells and are associated with increased ROS production. The common t(12;21)(p13;q22) chromosomal translocations occurs in 25% of paediatric B-cell precursor ALL (BCP-ALL) and result in a chimeric *ETV6/RUNX1* fusion gene [26]. *ETV6/RUNX1* translocation has been reported prenatally to initiate a pre-leukaemic state [27]. This “first-hit” incidence of *ETV6/RUNX1* fusion does not lead to malignant transformation; however, it can impair the function of wild type ETV6 and RUNX1, leading to cell cycle dysregulation [28]. Transgenic mouse models of ETV6/RUNX1 expressing $CD19^+$ B cells, showed increased ROS and increased DNA damage [29]. Although the exact mechanism by which ETV6/RUNX1 increases ROS are not known, ETV6/RUNX1 binds with the promoter region of

erythropoietin receptor (EPOR) to drive gene expression. Binding of EPO ligand with the EPOR in turn increases pre-B cell survival mediated by JAK2/STAT5 activity, which upregulates the antiapoptotic protein BCL-XL. Interestingly, NOX2-derived ROS in turn increases EPO signalling, suggestive of a positive feedback mechanism [30]. STAT5 binds and activates Rac1, promoting NOX mediated ROS production, to increase DNA damage in AML cell lines [31], a possible mechanism by which ETV6/RUNX1 induces ROS and garnishes the secondary hit necessary for preleukemic clones to propagate mutations that favour the development of fully transformed ALL.

2.2. BCR/ABL Oncogene

An alternative t(9;22)(q34;q11) translocation gives rise to the Philadelphia chromosome (Ph), which is present in approximately 3% of paediatric, and in 25% of adult B-ALL (Ph^+ B-ALL) [32]. This translocation produces the Breakpoint cluster-Abelson (BCR/ABL) fusion protein, in which the tyrosine kinase domain of ABL1 becomes constitutively active. Interestingly, BCR/ABL has also been reported in healthy individuals [33], identifying this event as a putative first hit, but can transform cells by itself. Accordingly, BCR/ABL fusion protein activates multiple ROS producing signalling pathways, including PI3K/Akt/mTOR and JAK/STAT [34] (**Figure 1**). PI3K/Akt in turn increases ROS production through increased mitochondrial activity and NOX activation, [35] generating a positive feedback loop. BCR/ABL driven ROS [36], induces DNA damage and drug resistance [37]. High-level of ROS production in leukaemic blasts harbouring recurring BCR/ABL oncogenes is regulated by Rac independent activation of NOX4. BCR/ABL driven ROS can also oxidise and inhibit the tumour suppressor serine threonine phosphatase PP1α leading to unfettered PI3K/Akt signalling and increased expression of proteins involved in cell survival [38]. The NOX and flavoprotein inhibitor diphenyleneiodonium (DPI), and tyrosine kinase inhibitors, Imatinib and Nilotinib, have both been shown to reduce ROS in preclinical models of BCR/ABL driven leukaemia [38][39]. Conversely, BCR/ABL transgenic mice express activated Rac3 in primary precursor B lymphoblasts, with molecular inhibition of *Rac3* increasing survival of mice harbouring BCR/ABL+ leukaemia [40]. As Rac3 plays an important role in the activation of NOX1-3 [41], it is likely that BCR/ABL mutations increase Rac3-NOX mediated ROS production in ALL.

Not only does BCR/ABL influence redox signalling, but it also enhances the ability of cells to survive DNA damage by increasing expression of RAD51 and decreasing expression DNA protein kinase- DNA-PK (*PRKDC)*, key elements of double-strand break (DSB) repair, homologous recombination repair (HRR) and non-homologous end-joining (NHEJ), respectively [42][43][44][45]. In cases of adult acute myeloid leukaemias (AML) driven by constitutive activation of the FMS-like tyrosine kinase 3 (FLT3) present in 30–35% of cases [46]; Leukaemias well characterised by redox dysfunction [25], increased activity of the NHEJ DSBs repair pathway is also seen and regulated by the phosphorylation of S2612 DNA-PK [47].

2.3. Cytokine Receptor-like Factor 2 (CRLF2)

Approximately 20% of B-ALL patients harbour an activated kinase gene expression profile resembling Ph^+ B-ALL but are negative for the BCR/ABL fusion [32]. Cytokine receptor-like factor 2 (*CRLF2*) encodes the receptor for thymic stromal lymphopoietin (TSLP), which activates cellular signalling on binding, required for normal lymphocyte function [48]. *CRLF2* rearrangements lead to the overexpression of *CRLF2* mRNA and protein [49] and are common

in patients diagnosed with 'Ph-like' B-ALL [50]. Increased minimal residual disease (MRD) is frequently seen in Ph-like ALL patients following upfront standard induction chemotherapies, giving rise to disease relapse and poor overall survival [51]. Activation of CRLF2 stimulates PI3K/Akt/mTOR and JAK/STAT signalling to deregulate lymphoid progenitor differentiation, increases metabolism and, drives ROS production and associated genomic instability [52][53] (**Figure 1**). These studies suggest that CRLF2 induced ROS production can positively regulate its levels by constitutive activation of JAK/STAT signalling. Interestingly, the majority of Down syndrome (DS) patients who develop ALL (62%) harbour increased CRLF2 expression, with genomic signatures enriched for increased DNA damage [54]. Approximately 50% of Ph-like B-ALL patients with *CRLF2*-rearranged also harbour *JAK1* or *JAK2* mutations [49][55]. Overexpression of *Crlf2* and mutant *Jak2* drives factor-independent cell growth of murine BaF3 pro-B cells [54]. Furthermore, a recurring gain-of-function mutation in *CRLF2* (F232C) seen in ALL patients, promotes constitutive dimerisation of the receptor, which may be induced by ROS, leading to cytokine independent activation and cell survival [56].

2.4. Interleukin-7 Receptor α (IL7R)

The Interleukin-7 receptor α (*IL7R*) is required for normal lymphoid development. Somatic gain-of-function mutations in *IL7R* are seen in paediatric B and T -ALL. A S185C substitution in the extracellular domain or in-frame insertions and deletions in the transmembrane domain constitutively activates the IL7R [57]. *IL7R* mutations are dominantly linked with the aberrant expression of CRLF2, where mutant IL7R forms a functional receptor with CRLF2 for TSLP. Hence, IL7R signalling drives ROS production and redox dysfunction through the activation of PI3K/Akt/mTOR, JAK/STAT and ERK/MAPK pathways, all of which are implicated in ALL disease progression and treatment resistance [58][59][60].

2.5. Transcription Factors PU.1 (SPI1) and Spi-B (SPIB)

The E26-transformation-specific (ETS) transcription factors PU.1 (*SPI1*) and Spi-B (*SPIB*) are important tumour suppressor genes that regulate B cell development and function [61][62]. Conditional deletion of *Spi1* and *Spi1B* in pre-B-cells impairs B cell differentiation and drives ALL development through ROS production and acquisition of secondary driver mutations in *JAK1, JAK3 or IKZF3* (IKAROS Family Zinc Finger 3). Acquired *JAK* mutations can further promote leukaemia progression through hyperactivation of JAK/STAT signalling and further increased ROS production driving DNA damage [63]. Conversely, the JAK inhibitor, ruxolitinib, delayed leukaemia onset and suppressed both ROS production and ROS associated gene expression signatures [64]. Decreased levels of PU.1 increase HSCs cell cycle progression through the activation of the MAPK pathway [65]. MAPK increases the activity of Activator protein 1 (AP-1), a transcription factor driving expression of the NOX activating subunit $p22^{phox}$ creating a feed-forward loop [66].

2.6. Neurogenic Locus Notch Homolog Protein 1 (NOTCH1)

NOTCH1 signalling is the most (70–80%) deregulated pathway in T-ALL [15]. NOTCH1 is a cell membrane receptor that plays a key role in the proliferation, differentiation, and activation of T cells. Mutations and constitutive activation of NOTCH1 in T-ALL promotes ROS production and PI3K/Akt signalling pathways indirectly through the

regulation of C-myc [67], which in turn induces DNA damage [68] (**Figure 1**). PTEN, an upstream negative regulator of PI3K/Akt/mTOR suppresses and hence decreases ROS production. Importantly, ROS induce redox PTMs and inactivation of PTEN's tyrosine phosphatase activity leading to sustained activation of PI3K/Akt/mTOR and thus ROS production through a positive feedback mechanism in T-ALL (**Figure 1**). Furthermore, Casein kinase 2 (CK2), which positively regulates the PI3K/Akt/mTOR pathway, is also elevated in NOTCH1 mutant T-ALL, further increasing ROS production and hyperactivation of PI3K/Akt/mTOR signalling. It follows that combinations of the NAC antioxidant and the CK2 inhibitor tetrabromobenzotriazole (TBB) can synergistically ablate NOTCH1 driven T-ALL survival [69].

2.7. Ras GTPases (N- and KRAS)

Activating mutations in either *N-* or *KRAS* are reported in 15–20% the paediatric ALL [70][71][72], and are enriched in relapsed and chemo resistant ALL patients [73]. Increased expression or activity of RAS guanyl-releasing protein 1(RASGRP1), a positive regulator of RAS/Raf/MEK pathway also occurs in ALL [74][75]. RAS proteins function as molecular switches that cycle between active and inactive states based upon the binding of guanosine triphosphate (GTP) and guanosine diphosphate (GDP), respectively. Normally, ligand mediated RTK activation is required to stimulate RAS and initiate a cascade of Raf/MEK signalling responsible for regulating multiple cellular functions including protein trafficking, ROS production and cellular proliferation [76][77]. However, mutations in codons 12, 13 and 61 impair the GTPase activity, constitutively activating RAS proteins and hence the downstream Raf/MEK pathway driving tumour growth [78]. Through the stimulation of NOX, constitutively active N-Ras^{G12D} and H-Ras^{G12V} upregulate ROS production promoting growth-factor independent proliferation of human CD34^{+} haematopoietic cells. Mechanistically, K-RAS activates p38 MAPK initiating a cascade of PDPK1/PKCδ/p47^{phox}/NOX1 signalling for the generation of ROS and cellular transformation (**Figure 1**). Given the role of RAS in NOX-induced ROS and recurring *RAS* lesions [24][70][71][72], it is very likely that constitutively active RAS drives ROS production in ALL, and as such patients may benefit from therapies targeting this ROS production pathway. Indeed, MEK inhibition, a downstream target of RAS, synergises with prednisolone in the treatment of both RAS-mutant and wildtype ALL [79], potentially underpinned by reduced ROS production following RAS inhibition.

2.8. Rho-Family GTPases

The increased expression and activation of Rho GTPase family proteins RAC1-3 is common in ALL [40][80][81]. Like Ras, Rac GTPases also cyclically switch between GTP-bound active and GDP-bound inactive states. While Rac1 regulated the chemotactic response of ALL cells to chemokine SDF-1α (CXCL12) produced by stromal cells [82], molecular inhibition of Rac3 prolonged the survival of mice with BCR/ABL induced leukaemia [40]. Similarly, central nervous system metastatic pre-B leukaemia cells (SD1-cells) overexpress Rac2, and when pre-treated with the Rac inhibitor, NSC23766, prior to engraftment, delayed disease establishment and significant reduction in leukaemia burden in extramedullary organs and the cranium [80]. The tetraspanin family member, CD9, is also reported to activate Rac1 and form cytoplasmic extensions and homing of B-ALL cells in vivo and in vitro [83]. Although the direct role of Rac proteins in ROS production has not been discussed, their reported role in NOX1-3

activation [41][84] raises the prospect that they may contribute to NOX mediated ROS production in ALL; an intriguing possibility that warrants further exploration.

2.9. NAD(P)H Quinone Dehydrogenase 1 (NQO1)

The NAD(P)H:quinone oxidoreductase 1 (NQO1) is a cytosolic two-electron oxidoreductase, detoxification and cytoprotective enzyme that protects cells against oxidative stress [85][86]. This enzyme functions as a superoxide reductase to generate antioxidant forms of ubiquinone and vitamin E to protect cells against oxidative stress. A polymorphism (C609T) in the *NQO1* gene leads to loss of protein expression, and hence loss of downstream ROS detoxification, with heterozygous (C/T) and homozygous (T/T) alleles reducing and abolishing reductase activity, respectively [87][88][89]. Studies report the increased association of low/null NQO1 activity and increased risk of infant ALL [88][90]. The loss/reduction in reductase activity is potentially associated with in utero genotoxic stress elicited through various oxidative cascades that accompany foetal haematopoiesis in the yolk sac from where it migrates to form part of the foetal bone marrow [91]. Conversely, overexpression of NQO1 is cytoprotective, and known to drive metastasis [92], with overexpressed NQO1 commonly detected in late stage and poor prognoses cancers [93]. Depletion of *NQO1* sensitised treatment-recalcitrant non–small cell lung cancers to low-dose radiotherapy by decreasing the cells reductase activity and increasing the genotoxicity of ionising radiation [94]. Furthermore, knockdown of *NQO1* in immune checkpoint inhibitor resistant tumours increased innate immune response to stimulate antitumour T cell adaptive immunity, and when retreated with checkpoint blockade therapies, eradicated therapy-resistant tumours [95].

3. Redox Homeostasis in ALL

ALL cells have evolved several compensatory mechanisms to ensure that ROS production does not induce irreversible DNA damage and cell death [96] (**Figure 1**). Many of these mechanisms centre on the expression of antioxidant systems with, for example, the gene and protein levels of thioredoxin reductase 1 (TXNRD1), TXN1 and PRDX1 all being elevated in B-ALL cell lines and primary patient samples [97]. Similarly, although H_2O_2 is usually a key signal in dexamethasone-induced apoptosis, pre-B-ALL cells that display dexamethasone resistance are characterised by overexpression of GSH; yet can be re-sensitised using L-buthionine-(S, R)-sulfoximine (BSO), an inhibitor of GSH synthesis [98]. Furthermore, thymic lymphoma cells that overexpress catalase are also resistant to dexamethasone [99]. As previously noted, manganese superoxide dismutase (MnSOD) overexpression drives H_2O_2 production and thus sensitises thymic lymphoma cells to dexamethasone via the release of mitochondrial cytochrome c and activation of caspases [100]. Importantly, increased GSH expression is associated with the increased risk of relapse in children diagnosed with ALL [101]. High-level expression of *GPx1* is also seen in ALL, influenced by decreased expression of *miR-491-5p* and *miR-214-3p* via VPS9D1 antisense RNA 1 [102]. Knockdown of *GPx1* reduced proliferation and activated apoptosis, with the *VPS9D1* antisense RNA 1 acting as a tumour promoter to increase *GPx1* expression and decrease *miR-491-5p* and *miR-214-3p*.

Regardless of the subtype, nuclear factor-κB (NF-κB) complexes show constitutive activation in paediatric ALL [103][104]. NF-κB proteins are a family of transcription factors that regulate immune responses to pathogens,

inflammation, promote growth and proliferation, and cell development [105]. Indeed, NF-κB transcription factors are responsible for the expression of the ROS producing NADPH oxidase enzymes [106] but can also upregulate the expression of antioxidants (reviewed in [107]). Not only does NF-κB activity increase NADPH oxidase expression and hence ROS production, but ROS regulates the transcriptional activity of NF-κB through degradation of the NF-κB inhibitory protein IκB, promoting nuclear translocation and transcription of κB genes, creating both a feed-forward and positive feedback loop [108][109]. Potentially, the chronic activity of PI3K-Akt signalling driven by recurring ALL associated somatic mutations (BCR/ABL, *IL7R/CRLF2*), may help to initiate the NF-κB positive feedback loop. Akt activates IκBα kinase (IKK) and p38 MAPK leading to the phosphorylation and degradation of IκB [110]. Loss of PTEN expression further potentiates NF-kB activity through unfettered PI3k/Akt signalling driving the activity of IKK, and further degradation of IκB, supressing apoptosis [111] and driving resistance to doxorubicin [112].

The observations that NF-κB acts an oncogene in ALL, contrasts with pre-B-ALL studies suggesting NF-κB1 is a tumour suppressor. The ratio of phosphorylated and hence nuclear translocated STAT5 to RELA expression (NF-κB transcriptional effector) correlates with B-ALL patient survival and disease remission [113]. Competition between STAT5 and NF-κB for common binding sites increased expression of STAT5 genes including Cyclin D2 and D3 (*CCND2, CCND3*) and the oncogene MYC (*MYC*) [113], driving an aggressive form of B-ALL. As there is no doubt that STAT5 plays a critical role in the leukaemogenesis of B and T -ALL, with its activity required for transformation downstream of ALL oncogenes [114][115][116], it is interesting to postulate the direct roles ROS plays in the activity of STAT5 and vice versa. Like NF-κB, STAT5 enhances transcription of the NOX (specifically NOX4). By doing so, STAT5 promotes increased ROS production which acts as a feed-forward loop. However, it is unknown whether the activity of STAT5 plays a direct role in NOX complex activation in ALL. In AML, phosphorylated STAT5 has also been shown to co-localise with Rac1, suggesting a mechanism in which phosphorylated STAT5 promotes ROS production by NOX. Given Rac1 is overexpressed in primary ALL and AML primary blasts compared to controls [117], and pharmacological inhibition of Rac1 is selectively cytotoxic to primary ALL cells and not on normal lymphocytic cells [118], it is indeed possible that the high-level activity of STAT5 in ALL may promote Rac1- induced NOX activity helping to form a feed-forward loop analogous to AML.

There is clear evidence of oxidative dysfunction in ALL. However, to ensure ROS accumulation does not exceed the tipping point and shift from their leukaemogenic roles to induction of regulated cell death pathways, these malignant cells hijack homeostatic mechanisms for survival. The Nuclear factor-erythroid factor 2-related factor 2-NRF2 (*NFE2L2*), a transcription factor negatively regulated by Kelch-like ECH-associated protein 1 (*KEAP1*) under basal conditions. ROS mediated redox cysteine PTMs induce conformational changes in KEAP1, leading to the release and subsequent translocation of NRF2 to the nucleus. Within the nucleus, NRF2 binds to antioxidant response element loci located at the promoters of multiple genes that orchestrate cytoprotection through rapid expression of *NQO1*, combatting oxidative stress and driving cell survival [119][120]. A recent study showed 73% of paediatric ALL patients (22/30) harboured nucleotide changes in genes mapping to the KEAP1/NRF2/NF-κB1/p62 pathway [121]. The significant functional crosstalk between NF-κB and NRF2 suggests that both play important roles in the oxidative dysfunction of ALL cells. It is interesting to note that ALL cells are afforded protection against standard induction chemotherapies via interaction with neighbouring adipocytes [122]. In this regard, daunorubicin

treatment of ALL cells has been shown to dramatically upregulate NRF2-mediated oxidative stress response in adipocyte co-cultures and protect ALL cells from genotoxic stress. Such data implies that ALL cells induce oxidative stress in adipocytes through yet an unknown mechanism. Blocking GSH synthesis in adipocytes subsequently re-sensitises ALL cells to daunorubicin, suggesting adipocyte secreted exogenous antioxidants protect ALL cells from chemotherapy [123]. In a similar context, mesenchymal stem cells (MSCs) found in bone marrow release thiols (antioxidant) to protect T-ALL cells from parthenolide induced oxidative stress [124].

In addition to intracellular factors, ALL cells have been reported to make direct contact with the bone marrow stromal cells via tunnelling nanotubes (TNTs); long cylindrical non-adherent actin-based cytoplasmic extensions that play an important role in direct communication and transfer of macromolecules between adjacent cells [125]. Recently, Jurkat ALL cells were reported to directly transfer mitochondria to the bone marrow stromal cells via TNTs upon exposure to chemotherapeutic drugs, thereby reducing ROS induced cellular death [126]. Furthermore, primary patient derived pre-B-ALL cells signal to the bone marrow stromal cells through TNTs, driving secretion of pro-survival cytokines such as interferon-γ–inducible protein 10/CXC chemokine ligand 10 (*CXCL10*), IL-8, and monocyte chemotactic protein-1/CC chemokine ligand (*CCL2*) causing resistance to prednisolone [127].

T-ALL switch their metabolic programs in a similar way to normal HSCs when cultured in low oxygen [128]. Reduced mitochondrial activity and cell cycle progression in these low oxygen niches increases glycolysis and lowers their sensitivity to vincristine and cytarabine (cell cycle-related drugs) and dexamethasone, compared with T-ALL cells grown under normoxic conditions [129]. While low oxygen levels suppressed the activity of mTORC1, it increased the activity of HIF1α with the concomitant increase in the expression of HIF1α effector genes such as, *VEGF, GLUT3* and *CXCR4* to reduce mitochondrial activity and as such ROS levels in ALL [129].

In contrast, B-ALL cells seem to rely more on oxidative phosphorylation and mitochondrial activity than T-ALL [130]. B-ALL cells with reduced NADP/NAD^+ ratios were enriched for functional leukaemia-initiating cells (LICs) resistant to cytosine arabinoside (Ara-C) [130]. These cells maintained their oxidative stress levels and resistance to Ara-C through the activation of pyruvate dehydrogenase complex component X (*PHDX*). Further, Ara-C resistance was attenuated by suppressing oxidative phosphorylation using venetoclax, metformin, and berberine, inhibitors of mitochondrial metabolism in vitro and in vivo [130].

Autophagy is another mechanism that aids leukaemic cells to survive oxidative stress-induced apoptosis. By way of example, ROS have been shown to induce the expression of Beclin-1 (*BECN1*) (an autophagy related protein with an essential role in autophagosome formation) and increase the removal of injured mitochondria to drive chemotherapy resistance in ALL [131][132]. Importantly, quinacrine (QC) (an anti-malaria drug that potently inhibits autophagy) in combination with vorinostat (a pan-histone deacetylase (HDAC) inhibitor), significantly increased ROS production, which reduced autophagy and caused synergistic apoptosis in T-ALL cells [133].

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