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Chapter

Reactive Oxygen Species and Metabolic Re-Wiring in Acute Leukemias

Andrew J. Robinson, Richard L. Darley and Alex Tonks

Abstract

Reactive oxygen species (ROS) is the collective term for several oxygen containing free radicals, such as hydrogen peroxide. ROS is important in innate immunity, protein folding in the endoplasmic reticulum and as a cell signalling molecule involved in cellular proliferation, survival, differentiation, and gene expression. ROS has been implicated in both hematopoietic stem cell quiescence and hematopoietic differentiation. Consequently, ROS is of considerable interest as a therapeutic target, with both pro-oxidant and anti-oxidant cellular modulation being explored. Recently, it has been established that increased ROS production in acute myeloid leukemia (AML) leads to increased glycolysis and metabolic reprogramming. It is often stated as a key tenet of the Warburg effect, that transformed cells, including AML, show increased aerobic glycolysis accompanied by increased cellular glucose uptake and lactate secretion. This review will summarize ROS state of the art in acute leukemia and how these reactive molecules re-wire metabolism in cancer cells. The review will focus on what are ROS? What are the sources of ROS in hematopoietic cells and their function and how this relates to the Warburg effect and regulation of metabolic pathways in acute leukemias.

Keywords: NADPH, NOX, ROS, hematopoiesis, HSC, AML

1. Introduction

To maintain hemostasis, new blood cells must be constantly generated to replace those lost through injury, disease, or age. Hematopoiesis, is the process where hematopoietic stem cells (HSC) differentiate into mature blood cells and is tightly regulated by the bone marrow (BM) micro-environment (or stem cell niche; reviewed in [1]), signal transduction pathways (reviewed in [2]), cytokines (reviewed in [3]), transcription factors (reviewed in [4]), epigenetics, (reviewed in [5]) and metabolic pathways (reviewed in [6]). HSCs are rare, constituting only 0.001% of peripheral blood (PB) and 0.05% of BM cells, but are responsible for producing a lifetime supply of blood cells. HSCs are cells that able to durably self-renew whilst also being multipotent. This differentiation is generally considered to occur via several intermediate progenitor cells, ultimately terminating in the specific mature blood cell through a process termed fate restriction or lineage commitment.

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The compartmentalization of HSC, their progenitors and terminally differentiated blood cells, into different stages of differentiation, is traditionally based on the expression of cell surface proteins (**Figure 1**). The recent emergence of single cell technologies such as fluorescent in situ hybridization, high-throughput single-cell quantitative PCR, single cell mass spectrometry and mass cytometry however, have led to re-analysis of these models of hematopoietic differentiation [7]. Discrete progenitor cell populations, as determined by cell surface markers, have been shown to consist of heterogenous populations with different fates [8]. Recently, a study by Velten *et al*., 2017, using a combination of single cell technologies and xenotransplantation as functional validation, proposed that early hematopoiesis consists of, a cellular continuum of low-primed undifferentiated (CLOUD) hematopoietic stem progenitor cells (HSPC), with simultaneous lineage gene expression for multiple fates [9]. This study suggested that early discrete stable progenitors do not exist, with any lineage determination occurring further downstream than originally presumed.

Regardless of provenance, leukemogenesis is characterized by a block in differentiation and an accumulation of immature white blood cell blasts with a rapid increase in these blasts, characteristic of the acute leukemias. Acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) are heterogenous diseases with a block in lymphoid or myeloid differentiation, respectively. They occur due to one or more genetic insults. Whilst ALL is predominantly a disease of children (80%), with a greater than 90% 5 y survival rate [10], in adults long term survival stands at only 30–40% [11]. AML in contrast is primarily a disease of the elderly, and like adult ALL it's 5 y survival rate is around 30%, however this falls in the over

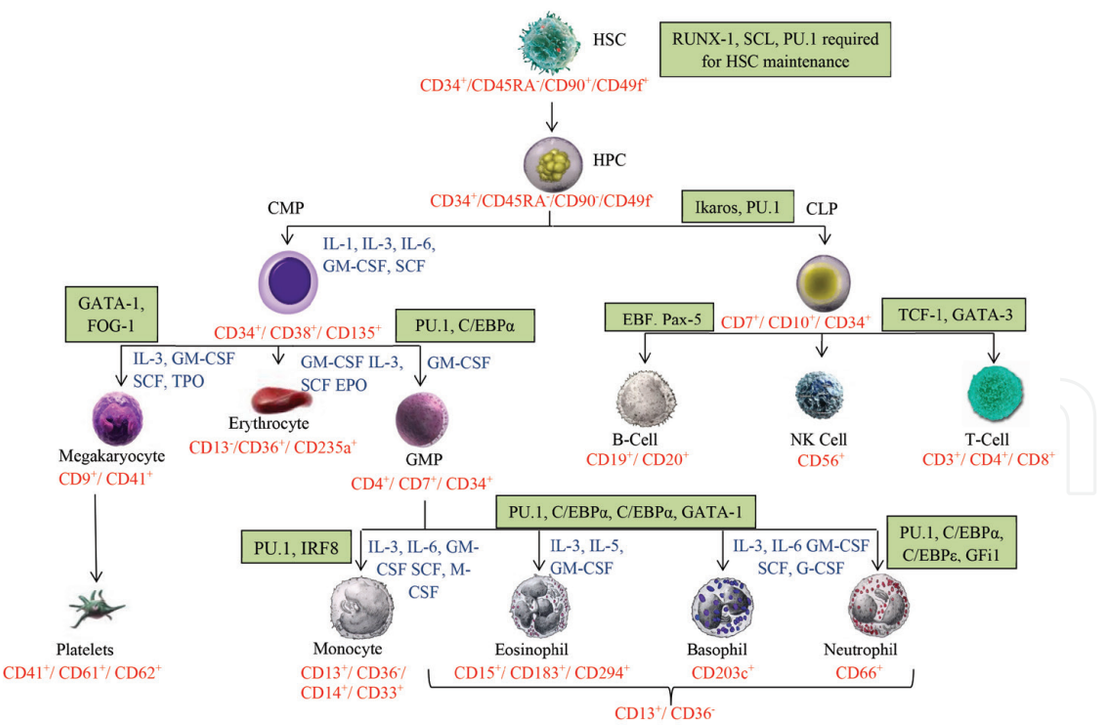

Figure 1.
Human hematopoiesis. Schematic diagram showing classical model of hematopoietic lineage commitment, with phenotypical cell surface markers (red), transcription factors determining differentiation (green box) and growth factors involved in myelopoiesis (blue). Hematopoietic stem cell (HSC), cluster of differentiation (CD), hematopoietic progenitor cell (HPC), common myeloid progenitor (CMP), common lymphoid progenitor (CLP), interleukin (IL), granulocyte macrophage (GM) colony-stimulating-factor (CSF), stem cell factor (SCF), thrombopoietin (TPO), erythropoietin (EPO), granulocyte myeloid progenitor (GMP), runt-related transcription factor 1 (RUNX1), transcription factor stem cell leukemia (SCL), ccaat enhancer binding proteins (C/EBP), friend of GATA protein 1 (FOG-1).

60's to a particularly bleak 10% [12]. In ALL, recent advances for example in the use of tyrosine kinase inhibitors and CAR-T cell therapy, have started to suggest improvements to overall survival [10]. However, in patients fit enough to tolerate chemotherapy, the standard treatment for AML since 1973 has been a seven-day continuous intravenous infusion of cytarabine (Ara-C) (100–200 mg/m^2) and 3 daily doses of daunorubicin (45–90 mg/m^2), sometimes followed by allogeneic or autologous stem cell transplantation, and despite some recent advances (reviewed in [13, 14]), current treatments appear to have reached their efficacious limits and new therapies are required.

One potential therapeutic opportunity involves exploiting the metabolic differences that exist between malignant and non-malignant cells [15]. Differences that, in AML at least, appear exacerbated by cellular levels of reactive oxygen species (ROS) [16].

2. Reactive oxygen species

ROS is the collective term for several oxygen containing free radicals and other reactive molecules, such as hydrogen peroxide (H_2O_2). Physiologically, ROS are initially generated via the univalent reduction of molecular oxygen which generates superoxide ($O2^{\bullet-}$). Superoxide (t1/2 = 1 μs) subsequently dismutates to H_2O_2 (t1/2 = 1 ms) [17], either spontaneously or via the catalytic action of the enzyme superoxide dismutase (SOD), or reacts with other ROS molecules, forming a variety of other ROS (**Figure 2**). Functionally, ROS is important in innate immunity,

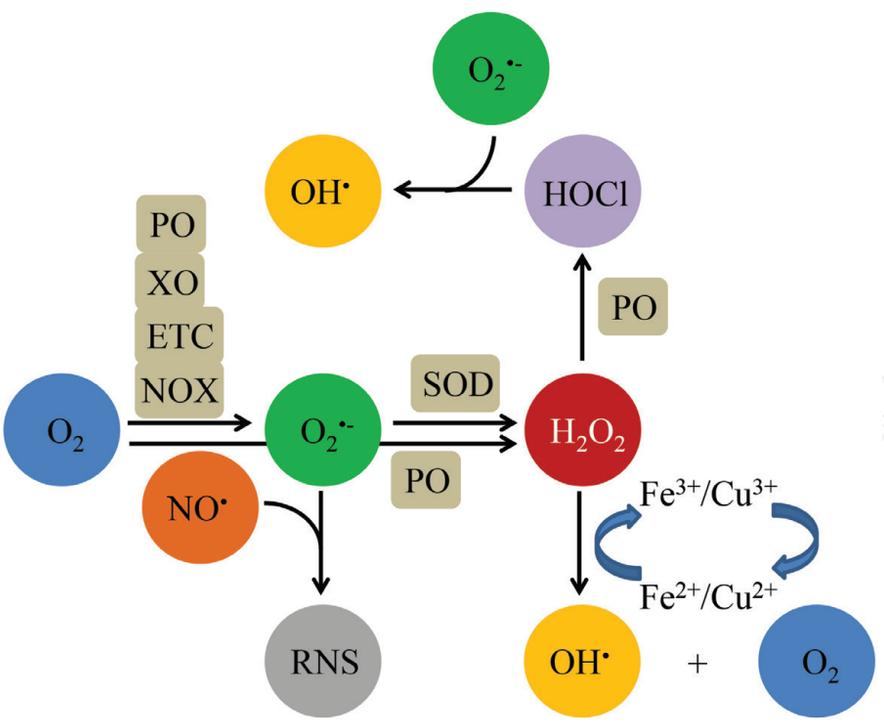

Figure 2.
Formation of reactive oxygen species (ROS). Diatomic oxygen (O_2) is univalently reduced by peroxisomes (PO), xanthine oxidase (XO), the electron transport chain (ETC), or NADPH oxidase (NOX) to generate superoxide ($O_2^{\bullet-}$). PO may also reduce O_2 directly to form H_2O_2. $O_2^{\bullet-}$ may then dismutate to H_2O_2 either spontaneously or through the enzymatic action of superoxide dismutase (SOD). Hydroxyl radicals ($OH^{\bullet}$) may then be formed from H_2O_2 via the formation of hypochlorous radical (HOCl) in the PO, or via Fenton chemistry. Reactive nitrogen species (RNS) may also be formed through the reaction of nitric oxide radical ($NO^{\bullet}$) with $O_2^{\bullet-}$.

protein folding in the endoplasmic reticulum and as a cell signalling molecule involved in cellular proliferation, survival, differentiation and gene expression [18].

There are several sources of cellular ROS, including the mitochondria, the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase family of enzymes (NOX), the cytochrome P450 enzymes, peroxisomes and the metabolic enzyme xanthine oxidase (XO).

2.1 Sources of ROS: electron transport chain

Generation of ROS by the mitochondria is primarily a function of 'electron leakage' from the electron transport chain (ETC), however, mitochondrial ROS may also be generated as a result of numerous enzymes including monoamine oxidase, cytochrome b5 reductase, glycerol-3-phosphate dehydrogenase, aconitase, pyruvate dehydrogenase and α-ketoglutarate dehydrogenase (reviewed in [19]). Mitochondrial ROS production resulting from the ETC generates $O_2^{\bullet-}$, and is thought to occur as result of one of three mechanisms. The first mechanism is a consequence of a high NADH/NAD^+ ratio, and results from oxygen interacting with fully reduced FMN. Mitochondrial ROS generated by this mechanism has been observed due to mitochondrial mutation, physiological damage such as ischemia or aging, and only small amounts of ROS are thought to be generated via these mechanisms in normally respiring cells [20]. The second mechanism occurs when there is a high level of reduced co-enzyme Q (CoQH2) in complex II, which in the presence of a high proton motive force generated by the proton pump, force electrons back into complex I in a process known as reverse electron transport (RET). Whilst RET generated ROS has also been implicated in diseases such as ischemia, it is now also thought to be involved as a cell signalling molecule in metabolic adaptation, myeloid differentiation and response to bacterial infection [21]. The third mechanism of ROS generation by the ETC occurs at complex III and has also been implicated in ROS signalling. The formation of $O_2^{\bullet-}$ occurs at the ubiquinol oxidation centre (Qo) site of the cytochrome bc1 complex, in which fully oxidized CoQ supports formation of $O_2^{\bullet-}$, through the transfer of electrons from reduced heme b1 to molecular oxygen [22]. Generation of $O_2^{\bullet-}$ by complex I and II occurs exclusively in the mitochondrial matrix, whereas $O_2^{\bullet-}$ generated by complex III also occurs in the intermembrane space. $O_2^{\bullet-}$ generated in the mitochondrial matrix is rapidly converted to H_2O_2 by mitochondrial SOD (Mn-SOD), whereas $O_2^{\bullet-}$ generated in the intermembrane space travels through the outer mitochondrial membrane prior to conversion to H_2O_2 by cytosolic SOD (Cu/Zn-SOD).

2.2 Sources of ROS: nicotinamide adenine dinucleotide phosphate (NADPH) oxidase family of enzymes (NOX)

Whilst mitochondrial oxidative phosphorylation is a major source of intracellular ROS, the main source of extracellular ROS involves the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase family of enzymes (NOX). The NOX family of enzymes comprise of seven members, NOX1–5 and dual oxidase (DUOX) 1 and 2. NOX enzymes are transmembrane proteins that transfer electrons from NADPH to molecular oxygen, generating $O_2^{\bullet-}$ (or H_2O_2), which can then be converted to other forms of ROS. Different NOX isoforms share conserved structural features comprising of six helical transmembrane domains (TM) (with helix III and helix V containing two heme-binding histidines), and a C-terminus cytosolic domain (DH), which allows binding of FAD and NADPH (**Figure 3**). Difficulties in obtaining suitable levels of NOX proteins mean that to date relatively little crystal structure data is available. However, a recently published report [23], has

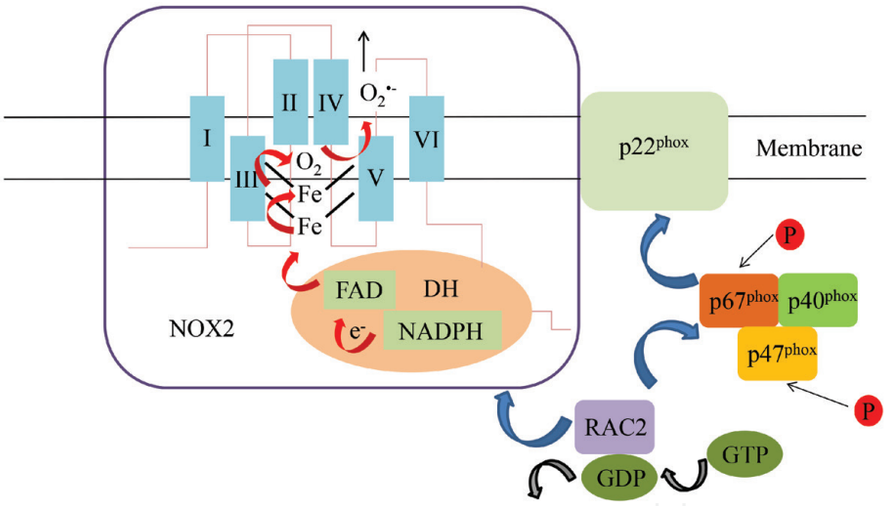

Figure 3.
Generation of superoxide ($O_2^{\bullet-}$) by NADPH Oxidase (NOX). Schematic diagram showing the major structural features of NOX2, it's activation by phosphorylation (P) of p67phoxand p47phox and the assembly of the major subunits of the NOX complex, and the generation of superoxide via electron transfer from NADPH to flavin adenine dinucleotide (FAD) to heme groups to diatomic oxygen. Guanosine triphosphate (GTP), guanosine diphosphate (GDP), homology domain (DH), RAS-related C3 botulinum toxin substrate 2 (Rac2).

elucidated the structure of the TM and DH domains (common to all NOX isoforms) of *Cylindrospermum stagnale* NOX5 in complex with FAD. In this structure, the six transmembrane helices of TM domain form a pyramidal shape with the base on the cytosolic side, a N-terminus α-helix runs parallel to the cytosolic side of the membrane and the heme groups sit in cavities formed by helices II–V, so that one is positioned near the cytosolic side of the membrane (heme 1) and the other on the outer side (heme 2). The DH domain, located on the cytosolic side, contains two pockets, one for FAD binding and one for NADPH binding. The FAD is positioned so that the flavin is in direct contact with heme 1 of the TM, to promote interdomain electron transfer. The proposed mechanism of electron transfer then involves NADPH donating its electron to FAD, which in turn donates an electron to heme 1 and then to heme 2 via Trp378 (equating to Phe 215 in human NOX2, Phe 200 in human NOX4 and Val 362 in human NOX5) of the loop between helix II and III of the TM, before reduction of molecular oxygen, via a final electron transfer step generates $O_2^{\bullet-}$ (**Figure 3**).

From a metabolic perspective, one source of NOX2 activation results when cells experience intermittent hypoxia. Under this condition activation of the metabolic enzyme XO, an enzyme important in the catabolism of purines and a major source of cellular ROS, occurs [24]. XO activation leads to increased ROS, which induces Ca^{2+} activation of protein kinase C, an enzyme important in cell signalling, migration of $p47^{phox}$ and $p67^{phox}$ to the cell membrane, resulting in activation of the NOX2 complex (**Figure 3**). Finally it is important to note, from a cell signalling perspective, that extracellular H_2O_2 (which is rapidly formed from $O_2^{\bullet-}$) is readily transported across the cell membrane via the transmembrane water permeable channel protein family of aquaporins [25, 26].

3. Role of ROS on normal hematopoiesis

ROS has been implicated in both HSC quiescence and hematopoietic differentiation. HSC reside in the bone marrow and their quiescence is known to be negatively regulated by ROS. Forkhead box O (FOXO) transcription factors are involved in

cell-cycle arrest and apoptosis and are activated in response to oxidative stress whereupon they translocate to the nucleus [27]. Translocation of FOXO4 to the nucleus has been shown to be a function of redox signalling, where oxidation of cys-239 by ROS mediates the formation of disulphide bonds with nuclear import receptor transportin-1, which in turn allows nuclear localization [28]. FOXO deactivation occurs as a result of phosphorylation in response to activation of the regulatory cell cycle PI3K/AKT/mTOR pathway, resulting in their export from the nucleus and subsequent degradation in the cytoplasm [29]. Studies in murine HSC have shown that deletion of FOXO3a, which upregulates transcription of Mn-SOD [30], results in decreased HSC renewal [31] which is mediated by the tumor suppressor protein ataxia-telangiectasia mutated (ATM) and is accompanied by elevated ROS levels and myeloid lineage expansion [32]. Deletion of ATM in mice resulted in BM failure which was restored following treatment with antioxidants [33]. In a different study, isolation of murine HSC into ROS high and ROS low populations showed that the ROS low population maintained self-renewal capacity following serial transplantations, whilst the self-renewal capacity of the ROS high population was exhausted following the third serial transplantation. Treatment of the ROS high HSC with the antioxidant N-acetyl cysteine (NAC), the p38 inhibitor SB203508 or rapamycin (a mTOR inhibitor), restored self-renewal activity [34]. Interestingly, the ROS high population in this study also exhibited a decreased ability to adhere to cells containing calcium sensing receptors, whilst NOX generated ROS has additionally been implicated in osteoclast differentiation in human mesenchymal cells, further emphasizing a potential regulatory role of ROS, in the BM niche [35].

Whilst these increased ROS levels are associated with HSC losing quiescence, it has also been shown, in the human megakaryocytic cell line MO7e, that hematopoietic cytokines, such as granulocyte macrophage-colony stimulating factor, interleukin-3, stem cell factor and thrombopoietin all increase ROS levels [36]. In megakaryopoiesis, ROS has been shown to increase platelet production and maturation in the chronic myeloid leukemia (CML) cell line MEG-01 and primary human megakaryocytes [37], which in murine models is mediated by the transcription factor NF-E2 [38]. Following lineage commitment, megakaryocyte progenitors undergo endomitosis (chromosomal replication in the absence of cell division), which in murine cells is potentially mediated by NOX1-derived ROS [39]. In human HSC, NOX-derived ROS has also been shown to be crucial for megakaryocyte differentiation via activation of ERK, AKT and JAK2 signalling pathways [40], whilst another study revealed the importance of cytochrome P450 2E1-generated ROS in megakaryocyte differentiation in human HSC [41]. As noted above, increased ROS in HSC has been associated with expanded myelopoiesis. Interestingly, a recent study using murine CMP, showed that higher levels of ROS impeded megakaryopoiesis, instead directing differentiation of CMP into GMP [42]. Finally, ROS has also been shown to induce differentiation of the promonocytic cell line, U937, into macrophages [43], and the differentiation of primary human monocytes into dendritic cells [44].

4. Role of ROS on solid tumors and leukemia development

One of the first studies implicating ROS in carcinogenesis was performed in mice subcutaneously injected with C3H mouse fibroblasts, that had been previously cultured *in vitro* with neutrophils stimulated with 12-O-tetradecanoylphorbol-13-acetate (TPA) stimulated or unstimulated or with the ROS generating enzyme XO and hypoxanthine. In this study approximately 20% of mice treated with these cells developed tumors within 13–22 weeks compared to none of the control mice [45].

In 1991, analysis of H_2O_2 production in human melanoma, colon, pancreatic, neuroblastoma, breast and ovarian cancer cell lines, revealed constitutively active H_2O_2 production over a 4 h period, generating H_2O_2 levels similar to those observed in TPA stimulated neutrophils, suggesting increased ROS production may be a feature of transformation [46]. Later, studies in patients with liver disease suggested ROS plays a part in hepatocarcinogenesis [47], and levels of Cu/Zn-SOD are significantly lower in hepatoma tissue than normal human liver tissue [48]. Further, homozygous deletion of Cu/Zn-SOD in mice results in decreased lifespan, with 70% developing hepatocarcinoma or benign nodular hyperplasia [49], whilst homozygous deletion of Mn-SOD in mice is lethal within two weeks of birth [50]. In the same study, heterozygous deletion of Mn-SOD resulted in increased incidence of hemangioma and adenocarcinoma and significant increases in the incidence of lymphoma. Currently, elevated ROS levels have been reported in many solid tumors and the role they play in tumorigenesis is complex and multifaceted (reviewed in [51]).

In leukemia, a study which collected blood samples from ALL and CML patients samples and compared them with normal blood samples showed elevated levels of ROS in both ALL and CML patients [52], whilst elevated levels of NOX generated ROS, are observed, alongside increased proliferation in both AML models and AML patient samples when compared with healthy controls [53]. Reactions of ROS with DNA can generate numerous oxidised bases, including 8-hydroxy-2-deoxyguanosine (8-OHdG) which causes G:C to T:A DNA transversions (reviewed in [54]). Increased levels of 8-OHdG have been observed in patients with breast cancer [55], gastric carcinomas [56], lung cancer [57] and colorectal cancer [58]. In leukemia, a study of 116 Chinese children with either ALL or AML revealed significantly elevated levels of 8-OHdG, whilst 8-OHdG levels were also significantly elevated in relapsed AML adult patients [59].

As a signalling molecule, ROS can lead to hyperactivation of the PI3K pathway, a common feature of many cancers, resulting in increased cell survival, VEGF production, secretion of MMP (reviewed in [60]) and inactivation of FOXO [32]. In AML, constitutive activation of the PI3K/AKT pathway is frequently observed [61, 62], however the role of FOXO is less clear. A recent study revealed that FOXO1 expression in osteoblasts mediated β-catenin initiated AML [63], whilst a study of AML patient samples showed that 40% exhibited FOXO activation, that upon inhibition resulted in myeloid differentiation and AML cell death [64]. Additionally, in both CML and AML the BCR-ABL fusion protein and FMS-like tyrosine kinase receptor 3 internal tandem duplications (FLT3-ITD) have been shown to lead to phosphorylation of AKT resulting in increased activation of NOX, and increased ROS production (reviewed in [65]), which may in turn reinforce PI3K/AKT activation.

5. Metabolism and cancer

Broadly defined, cellular metabolism involves a series of catabolic or anabolic chemical reactions which generate or use energy as part of this process. In chemotrophs this energy is obtained through the oxidation of nutrients, with the energy typically stored in the form of ATP. Whilst in higher organisms a plethora of enzymatically catalyzed metabolic reactions occur, which are all part of different interconnecting metabolic pathways with multitudinous feedback mechanisms. These pathways are evolutionarily highly conserved with the citric acid cycle, for example, essentially a feature in all terrestrial life. There are three main classes of molecules involved in metabolism; carbohydrates, proteins and lipids that are either catabolized to generate energy or energy stores or used by anabolic pathways

in the synthesis of, for example, nucleotides and structural molecules such as cell membranes. In mammals, a triumvirate of glycolysis, citric acid cycle and the ETC are central to the generation of ATP, with glycolysis and the citric acid cycle contributing 2 ATP molecules each and the ETC generating up to 34 ATP molecules in a process collectively termed aerobic respiration (reviewed in [66]).

Given the skew towards ATP production in the ETC, Otto Warburg's observation in 1956 that aerobic glycolysis was a hallmark feature of cancer cells [15], was initially attributed to being the result of defective mitochondria in malignant cells, and initially raised little interest. However, this hypothesis is now known in most cases to be incorrect (reviewed in [67]) and instead, it has been shown that mitochondrial respiration is often necessary in tumorigenesis [68]. However, given its ubiquity and despite its inefficiency when compared with ETC, it is clear that the phenomenon of increased aerobic glycolysis (eponymously titled 'The Warburg Effect'), must offer cancer cells some competitive advantage, although its exact ontology remains unclear. One hypothesis contends that whilst inefficient, aerobic glycolysis generates ATP at a rate 10–100 times faster than oxidative phosphorylation, therefore supplying cancer cells with energy at a faster rate. This increased glycolytic flux could then, potentially generate more nucleotides, amino acids and lipids for biosynthesis as well as generating the reducing agent NADPH, to deal with the increased levels of ROS common in many cancer cells [69]. Alternatively, increases in excreted lactate as a result of aerobic glycolysis would likely generate a more acidic microenvironment, breaking down stromal membrane structures and potentially increasing cancer cell motility and metastasis [70].

5.1 NADPH: a link between ROS and metabolism in cancer

It has been shown that activation of the tumor suppressor protein ATM by ROS promotes glucose-6-phosphate dehydrogenase (G-6-PD) activity, the first step of the pentose phosphate pathway (PPP), which in turn generates NADPH [71]. Given that major cellular antioxidant systems, ultimately rely on NADPH to provide their reducing power, it is perhaps not surprising that ROS in both normal and aberrant cellular processes is inextricably linked with metabolism. In the cytosol, NADPH is primarily generated through the PPP, whilst a number of mechanisms exist for mitochondrial NADPH generation [72], which include the serine synthesis pathway (SSP) (via the folate cycle) [73] and the action of the citric acid cycle enzyme isocitrate dehydrogenase (IDH). IDH1 and IDH2 are commonly mutated in AML [74], although in this context NADPH is consumed, and the D-2-hydroxyglutarate generated leads to stabilization of the hypoxia regulator, hypoxia inducible factor alpha (HIF-1α) [75].

HIF-1α as a target of ROS is controversial [76], however it is overexpressed in many cancers where it induces expression of numerous glycolytic genes. The ROS regulated transcription factor nuclear-related factor 2 (NRF2) has also been shown to modulate metabolism in lung cancer cell lines, through the upregulation of enzymes involved in the NADPH production, notably G-6-PD, IDH1 and malic enzyme 1 [77] and high NRF2 levels have previously been reported in AML [78]. Furthermore, the tumor suppressor protein TP53 is also important in regulating metabolism. Homozygous deletion of TP53 in mice results in decreased oxygen consumption arising from decreased mitochondrial respiration [79]. TP53 expression has been shown to inhibit, both glucose transporter (GLUT) 1 and 4 and the glycolytic enzyme phosphoglycerate mutase (PGAM) (reviewed in [80]) leading to decreased glycolysis and potentially increased metabolism via the PPP and SSP. Finally, TP53 also upregulates the apoptosis regulator (TIGAR) an enzyme which has an active domain similar to 6-Phosphofructo-2-kinase/fructoste-2,6-bisphosphatase

(PFKFB). TIGAR catalyzes the reaction of fructose-2,6-bisphosphate (F-2,6-BP) to fructose-6-phosphate (F-6-P), which inhibits glycolysis, redirects metabolites into the PPP, generating NADPH [81].

5.2 ROS regulation of metabolic pathways

Changes of cellular ROS levels in both normal signalling as well cell signalling following cellular transformation result in changes in numerous signalling pathways controlling multiple cellular functions including growth, proliferation and differentiation. A number of these signalling pathways, exercise regulatory control over various metabolic pathways, which in turn modulate ROS levels via several feedback mechanisms (**Figure 4**). In leukemia, mutations in the *RAS* gene are present in about 15% of hematological malignancies [82]. RAS activates the PI3K/AKT/mTOR pathway which promotes nucleotide biosynthesis and lipid synthesis (reviewed in [83]) as well as HIF-1α, which upregulate glycolysis via the activation of numerous glycolytic genes. In addition to HIF-1α, other ROS activated transcription factors are important in metabolic regulation such as STAT3, which has been shown to promote glycolysis in hepatocellular carcinoma cell lines [84], FOXO3A, which inhibits glycolysis via activation of tuberous sclerosis 1 protein [85] and NF-κB which was shown to upregulate GLUT3 in mouse embryonic fibroblasts [86].

Nuclear localization of the glycolytic enzyme pyruvate kinase muscle 2 (PKM2) is also ROS mediated, where it acts as a co-factor in the activation of the transcription factor, c-MYC. RAS also activates c-MYC which is overexpressed in greater than 50% of human cancers and c-MYC has been shown to activate glycolysis via the upregulation of GLUT, the glycolytic enzymes hexokinase (HK),

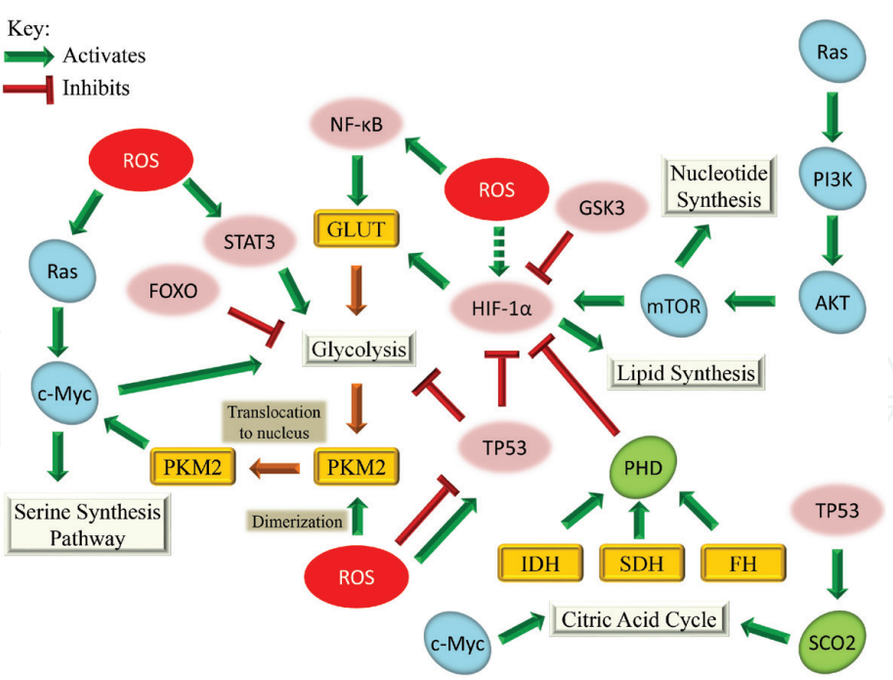

Figure 4.
Regulation of metabolic pathways. Schematic illustration outlining some of the regulatory mechanism involved in glycolysis and other key metabolic pathways. Transcription factors are in pink and signalling pathways in blue. Reactive oxygen species (ROS), forkhead box O (FOXO), pyruvate kinase muscle 2 (PKM2), signal transducer and activator of transcription (STAT), nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB), glucose transporter (GLUT) hypoxia inducible factor-1 alpha (HIF-1α), tumour suppressor protein 53 (TP53), glycogen synthase kinase 3β (GSK-3β), isocitrate dehydrogenase (IDH), succinate dehydrogenase (SDH), fumarate hydratase (FH), protein kinase B (AKT), mammalian target of rapamycin (mTOR), phosphoinositide 3-kinase (PI3K), synthesis of cytochrome c oxidase 2 (SCO2) and prolyl-hydroxylase domain (PHD).

phosphoglucose isomerase (PGI), phosphofructokinase (PFK), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), PKM2, as well as lactate dehydrogenase A (LDHA), pyruvate dehydrogenase kinase 1 (PDK1) and PFKFB3 (reviewed in [87]). Increased glutaminolysis is also a target of c-MYC, which upregulates the glutamine transporter ASCT2 and a key enzyme glutaminase. Additionally, c-MYC was shown to upregulate both phosphoglycerate dehydrogenase (PHGDH) which catalyzes the first step of the SSP, serine hydroxymethyltransferase, part of the folate cycle as well as several genes involved in fatty acid metabolism and the citric acid cycle (reviewed in [67]). In contrast TP53 is known to inhibit glycolysis through inhibition of GLUT1, GLUT4 and PGAM and through activation of TIGAR and synthesis of cytochrome c oxidase 2 (SCO2). Inhibition of glycolysis also occurs due to the regulatory role of miRNA. For example, miR-195-5p inhibits GLUT3, miR-143 inhibits HK2 and miR-155 inhibits HIF-1α. Furthermore, TP53 induces miR-34a which suppresses HK1, HK2, GPI and PDK1, as well as sirtuin 1, which activates FOXO1, NF-κB and in a positive feedback loop TP53 (reviewed in [80]).

5.3 Metabolism and leukemia

Given the role that ROS plays in regulating metabolism, it is not surprising that expression of nearly all enzymes associated with glycolysis have been shown to be altered in solid tumors, a pattern also observed in leukemia. In ALL, micro-array analysis showed significant upregulation of PFK as well as the glucose transporters GLUT1 and GLUT4 in pediatric B-ALL samples [88], whilst deletion of GLUT1 in primary human B-ALL cells suppressed leukemic progression *in vivo* [89]. In AML, upregulation of *GLUT1* mRNA [90] and the fructose transporter *GLUT5* [91] have also been reported to be associated with poor outcome in AML patients. Furthermore, NOX generated ROS has previously been reported to modulate cellular glucose uptake through increased GLUT1 activity, in leukemic cell lines [92]. In Philadelphia$^+$ ALL (Ph$^+$ALL) GLUT5 has been found to be upregulated at both the mRNA and protein level [93]. Song *et al* have identified HK2 overexpression as a feature of AML patients who failed to show remission [90], whilst decreased proliferation in the AML cell line, KG-1, was observed upon knock-down of PGI with shRNA [94]. The HK inhibitors 2-deoxy-D-glucose and 3-bromopyruvate have both been shown to be cytotoxic in AML patient samples harboring a FLT3-ITD mutation both alone and in combination with sorafenib [90, 95]. In chronic lymphocytic leukemia (CLL), a study by Ryland et al., 2013 showed increased expression of glyceraldehyde phosphate dehydrogenase (GAPDH) in CLL patients compared to healthy controls [96]. Proteomic studies revealed elevated levels of aldolase A (ALDO(A)), ALDO(C) and enolase 1 (ENO1) in the chemoresistant leukemia cell line K562/A02 when compared with parental K562 cells and in the case of ENO1 this was confirmed by western blot [97]. Elevated levels of ENO2 have also been reported in patients with ALL where it is associated with lower overall survival [98], whilst PGAM is upregulated in both AML and CML patient samples [99]. LDH is a tetramer which exists as five isoforms, comprising of two subunits LDHA and LDHB in different combinations and encoded by the LDHA and LDHB genes [100], with LDHA strongly catalyzing pyruvate to lactate and LDHB preferentially catalyzing the reverse reaction. In B-ALL, mRNA expression levels of *LDHB* were shown to be decreased [88], suggesting increased lactate production, whilst more recently increased serum levels of LDH were found in patients with B-ALL in conjunction with increased levels of total oxidant status and decreased total anti-oxidant status [101]. Another recent study involving 204 patients with acute leukemia's also reported that LDH plasma levels were significantly elevated compared to healthy

controls and were also increased in relapse patients compared to those in complete remission [102]. Recently, it was shown that ROS dependent proliferative increases observed in hematopoietic models [103] were also accompanied by increased glucose uptake and expression of the regulatory glycolytic enzyme PFKFB3 [53], whilst downregulation of this enzyme suppressed growth both *in vivo* and *in vitro* [16]. This study also reported that metabolomic analysis comparing AML patient samples with high/low levels of ROS, which showed significantly elevated levels of glucose, glucose-6-phosphate (G-6-P) and F-6-P in the ROS high patients. Another metabolomic study involving serum from 400 AML patients compared with 446 healthy controls, identified elevated levels of the glycolytic intermediates 3-phosphoglycerate (3-PG), pyruvate and lactate as conferring a poor prognosis for survival [104]. Interestingly, a recent study showed that the bromodomain and extra-terminal protein inhibitor JQ1, which has shown promise in ALL by targeting c-myc, downregulates expression of HK2, PKM2 and LDHA both at the transcriptional and protein level [105].

The citric acid cycle is a series of metabolic reactions involving oxidation/reduction reactions, which generate nicotinamide adenine dinucleotide (NAD)H and flavin adenine dinucleotide (FAD)H via the transfer of hydride ions, thus providing electrons for the ETC which is a major source of cellular ROS (reviewed in [106]). Mutations of IDH, which catalyzes the decarboxylation of isocitrate to alpha-ketoglutarate are frequently reported in AML (reviewed in [107]). Characterization of the inhibitor AG-221, which has been shown to inhibit mutant IDH2 in AML cells *in vitro* and *in vivo* and is currently undergoing phase I/II clinical trials [108], as is the IDH1 inhibitor, AG-120 [109]. A metabolomic study which examined a cohort of 183 patients with *de novo* AML matched with 232 healthy controls showed significant differences in citrate levels between AML patients and controls [110]. In pediatric ALL a recent metabolomic study revealed increased metabolites of glycolysis, the citric acid cycle and the PPP in patients testing positive for measurable residual disease compared to those testing negative [111]. Interestingly use of nicotinamide phosphoribosyltransferase (NAMPT) inhibitors on ALL cell lines and patient samples showed cytotoxicity *in vitro*. NAMPT is a key enzyme in the synthesis of the oxidizing agent NAD^+, in both glycolysis and the citric acid cycle.

The SSP branches from the glycolytic pathway at the glycolytic intermediate 3-PG, where it is converted into 3-phosphohydroxypyruvate by the enzyme PHGDH, followed by conversion to phosphoserine by phosphoserine aminotransferase 1 and finally to serine by the action of the enzyme phosphoserine phosphatase (reviewed in [73]). Regulation of the SSP is achieved through 2-phosphoglycerate (2-PG) which activates PHGDH whilst serine activates the tetrameric form of PKM2 leading to increased glycolysis and decreased levels of 2-PG. Importantly serine can enter the folate cycle, which provides another route for the generation of NADPH, which has been shown to contribute to tumor growth *in vivo* [112]. Whilst overexpression of PHGDH has been reported in melanomas, colorectal and breast cancers, little has been published from a leukemia perspective. Knock-down of PHGDH has been shown to inhibit the growth of the leukemia cell line, HL-60 [113], and in multiple myeloma increased expression of PHGDH led to increased SSP activity and antioxidant capacity in cells resistant to treatment with the proteasome inhibitor bortezomib [114].

The PPP generate nucleotides for biosynthesis and is a major source of cellular NADPH, an important cellular antioxidant. The first step involves the dehydrogenation of G-6-P to 6-phosphogluconolactone (6-PG) catalyzed by G-6-PD and the conversion of $NADP^+$ to [115]. Gluconolactonase catalyzes the hydrolysis of 6-PG to 6-phosphogluconate, which is then catalyzed by 6-phosphogluconate dehydrogenase (6-PGD) to ribulose-5-phosphate (Ru-5-P) alongside the generation of a

second NADPH. Ru-5-P can then be converted into ribose-5-phosphate (R-5-P) by the enzymatic action of ribulose-5-phosphate isomerase. R-5-P can then be used in the synthesis of nucleotides. Alternatively, where redox homeostasis and not nucleotide synthesis is the major requirement of the cell Ru-5-P can be catalyzed by ribulose-5-phosphate epimerase, into xyulose-5-phosphate (X-5-P) and via a series of further metabolic reactions back into the glycolytic intermediates F-6-P and glyceraldehyde-3-phosphate. G-6-PD is the rate limiting step of the PPP and is regulated by the NADP+/NADPH ratio, RAS/PI3K signalling and phosphorylation by Src, whilst 6-PGD is inhibited by 3-PG [99]. In cancer, aberrant RAS signalling or activation of Src can promote activation of the PPP. In AML, a recent study showed upregulation of *G-6-PD* mRNA in approximately 60% of patients, although it was not correlated with overall survival or relapse [116]. Targeting of xenograft mice injected with the leukemic cell line K562, with the antimalarial drug dihydroartemisinin and the 6-PGD inhibitor Physicon resulted in decreased tumor growth, whilst primary leukemia cells isolated from the PB of AML patients showed significantly decreased viability, with no toxicity observed in hematopoietic cells isolated from healthy individuals [117]. A metabolomic study comparing primary AML samples with either high or low levels of ROS, have also shown increased levels of the PPP metabolites sedoheptulose-7-phosphate and Ru-5-P in the samples with higher ROS levels [16]. Another study, using both AML cell lines and patient material, showed increased glucose metabolism and increased flux through the PPP, alongside increased *G-6-PD* mRNA expression [118]. Importantly, this study showed that use of the G-6-PD inhibitor 6-aminonictoinamide (6-AN) in AML cell lines induced both *in vitro* and *in vivo* cytotoxicity, and induced apoptosis in primary AML cells but not normal HPCs. In B-ALL, redirection of carbon from the glycolytic pathway to the PPP by the serine/threonine-protein phosphatase 2A (PP2A), has been shown to occur to combat cellular oxidative stress. Synergistic inhibition of G-6-PD by 6-AN and PP2A inhibitor LB100 induced cell death in patient derived Ph^{+}ALL [119].

Lipid metabolism has also been shown to be dysregulated in both solid tumors and hematological malignancies (reviewed in [120]). Increased fatty acid oxidation (FAO) allows cancer cells to overcome metabolic and oxidative stress through the generation of ATP and NADPH. Significant changes to lipid metabolite levels are seen in AML patient samples with either high levels or low levels of ROS [16], whilst suppression of NOX2 has also been shown to increase FAO [121]. Furthermore, inhibition of the FAO using Avocatin B results in decreased NADPH levels and ROS dependent cell death in primary human AML samples but not normal mononuclear cells [122]. In ALL, use of L-asparaginase has been shown to increase FAO activity as a metabolic escape mechanism, however use of the FAO inhibitor etomoxir in combination with L-asparaginase has been shown to increase sensitivity of both leukemic cell lines and patient samples [123].

6. Conclusions

In the last twenty years, it has become increasingly clear that ROS play a significant role in cellular signalling, particularly pathways associated with growth, differentiation and survival, whilst its roles in HSC quiescence and normal hematopoiesis have started to be delineated. In many cancers including hematological malignancies, ROS levels have been shown to be elevated, leading to aberrant signalling in these pathways. Previously, arguments for both the use of anti-oxidant and pro-oxidant treatments in leukemia have been made (reviewed in [124]). Despite the transformation of survival rates in patients with acute promyelocytic leukemia

using arsenic trioxide [125] cancer cells often upregulate the production of antioxidants, and downregulate pro-apoptotic pathways such as TP53, as a response to high ROS, allowing them to escape apoptosis. In addition, it has been shown that both cancer stem cells [126, 127] and leukemic stem cells [128] exhibit low ROS levels, suggesting that even if treatment with pro-oxidants eliminates the bulk of cancer cells, cancer/leukemic stem cells may survive and relapse occur. Conversely, studies involving the use of antioxidants in treatment and epidemiological studies of antioxidant use, have shown mixed results (reviewed in [129, 130]). Increasingly it is becoming apparent that increased levels of ROS are leading to changes in signalling pathways directly or indirectly controlling metabolism, as a mechanism for managing oxidative stress. Whilst, it has long been known that cancer cells exhibit greatly altered metabolism, only recently have the purposes behind this altered metabolism, started to be elucidated. Consequently, synergistic treatments involving the use of metabolic inhibitors, alongside classical treatments for leukemias are being explored. Future work, elucidating the intricate mechanisms governing the interplay between ROS and metabolism, alongside new and more specific metabolic inhibitors provide much promise for the future treatment of leukemia.

Acknowledgements

We are grateful to Blood Cancer UK for programmatic funding and to Tenovus Cancer Care for funding Andrew Robinson. We are grateful to Wellcome ISSF for funding aspects of ROS research. We are grateful for support from the NCRI AML trials cell bank and the AML patients for providing primary samples used in several of our studies.

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References

[1] Ho MS, Medcalf RL, Livesey SA, Traianedes K. The dynamics of adult haematopoiesis in the bone and bone marrow environment. British Journal of Haematology. 2015;**170**:472-486

[2] Luis TC, Killmann NM, Staal FJ. Signal transduction pathways regulating hematopoietic stem cell biology: introduction to a series of Spotlight Reviews. Leukemia. 2012;**26**:86-90

[3] Tarasova A, Haylock D, Winkler D. Principal signalling complexes in haematopoiesis: structural aspects and mimetic discovery. Cytokine & Growth Factor Reviews. 2011;**22**:231-253

[4] Wolff L, Humeniuk R. Concise review: erythroid versus myeloid lineage commitment: regulating the master regulators. Stem Cells. 2013;**31**:1237-1244

[5] Alvarez-Errico D, Vento-Tormo R, Sieweke M, Ballestar E. Epigenetic control of myeloid cell differentiation, identity and function. Nature Reviews. Immunology. 2015;**15**:7-17

[6] Oburoglu L, Romano M, Taylor N, Kinet S. Metabolic regulation of hematopoietic stem cell commitment and erythroid differentiation. Current Opinion in Hematology. 2016;**23**:198-205

[7] Ye F, Huang W, Guo G. Studying hematopoiesis using single-cell technologies. Journal of Hematology & Oncology. 2017;**10**:27

[8] Cvejic A. Mechanisms of fate decision and lineage commitment during haematopoiesis. Immunology and Cell Biology. 2016;**94**:230-235

[9] Velten L, Haas SF, Raffel S, Blaszkiewicz S, Islam S, Hennig BP, et al. Human haematopoietic stem cell lineage commitment is a continuous process. Nature Cell Biology. 2017;**19**:271-281

[10] Samra B, Jabbour E, Ravandi F, Kantarjian H, Short NJ. Evolving therapy of adult acute lymphoblastic leukemia: state-of-the-art treatment and future directions. Journal of Hematology & Oncology. 2020;**13**:70

[11] Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. Blood Cancer Journal. 2017;**7**:e577

[12] Estey E, Gale RP. Acute myeloid leukemia therapy and the chosen people. Leukemia. 2017;**31**:269-271

[13] Tamamyan G, Kadia T, Ravandi F, Borthakur G, Cortes J, Jabbour E, et al. Frontline treatment of acute myeloid leukemia in adults. Critical Reviews in Oncology/Hematology. 2017;**110**:20-34

[14] Winer ES, Stone RM. Novel therapy in Acute myeloid leukemia (AML): moving toward targeted approaches. Ther Adv Hematol. 2019;**10**:2040620719860645

[15] Warburg O. On the origin of cancer cells. Science. 1956;**123**:309-314

[16] Robinson AJ, Hopkins GL, Rastogi N, Hodges M, Doyle M, Davies S, et al. Reactive Oxygen Species Drive Proliferation in Acute Myeloid Leukemia via the Glycolytic Regulator PFKFB3. Cancer Research. 2020;**80**:937-949

[17] Bienert GP, Schjoerring JK, Jahn TP. Membrane transport of hydrogen peroxide. Biochimica et Biophysica Acta. 1758;**2006**:994-1003

[18] Corcoran A, Cotter TG. Redox regulation of protein kinases. The FEBS Journal. 2013;**280**:1944-1965

[19] Angelova PR, Abramov AY. Functional role of mitochondrial reactive oxygen species in physiology. Free Radical Biology & Medicine. 2016;**100**:81-85

[20] Murphy MP. How mitochondria produce reactive oxygen species. The Biochemical Journal. 2009;**417**:1-13

[21] Scialo F, Fernandez-Ayala DJ, Sanz A. Role of Mitochondrial Reverse Electron Transport in ROS Signaling: Potential Roles in Health and Disease. Frontiers in Physiology. 2017;**8**:428

[22] Drose S, Brandt U. The mechanism of mitochondrial superoxide production by the cytochrome bc1 complex. The Journal of Biological Chemistry. 2008;**283**:21649-21654

[23] Magnani F, Nenci S, Millana FE, Ceccon M, Romero E, Fraaije MW, et al. Crystal structures and atomic model of NADPH oxidase. Proceedings of the National Academy of Sciences of the United States of America. 2017;**114**:6764-6769

[24] Nanduri J, Vaddi DR, Khan SA, Wang N, Makerenko V, Prabhakar NR. Xanthine oxidase mediates hypoxia-inducible factor-2alpha degradation by intermittent hypoxia. PLoS One. 2013;**8**:e75838

[25] Bertolotti M, Bestetti S, Garcia-Manteiga JM, Medrano-Fernandez I, Dal MA, Malosio ML, et al. Tyrosine kinase signal modulation: a matter of H2O2 membrane permeability? Antioxidants & Redox Signaling. 2013;**19**:1447-1451

[26] Miller EW, Dickinson BC, Chang CJ. Aquaporin-3 mediates hydrogen peroxide uptake to regulate downstream intracellular signaling. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**:15681-15686

[27] Essers MA, Weijzen S, de Vries-Smits AM, Saarloos I, de Ruiter ND, Bos JL, et al. FOXO transcription factor activation by oxidative stress mediated by the small GTPase Ral and JNK. The EMBO Journal. 2004;**23**:4802-4812

[28] Putker M, Madl T, Vos HR, de RH, Visscher M, van den Berg MC, et al. Redox-dependent control of FOXO/DAF-16 by transportin-1. Molecular Cell 2013;49:730-42.

[29] Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, et al. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell. 1999;**96**:857-868

[30] Kops GJ, Dansen TB, Polderman PE, Saarloos I, Wirtz KW, Coffer PJ, et al. Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. Nature. 2002;**419**:316-321

[31] Miyamoto K, Araki KY, Naka K, Arai F, Takubo K, Yamazaki S, et al. Foxo3a is essential for maintenance of the hematopoietic stem cell pool. Cell Stem Cell. 2007;**1**:101-112

[32] Tothova Z, Kollipara R, Huntly BJ, Lee BH, Castrillon DH, Cullen DE, et al. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. Cell. 2007;**128**:325-339

[33] Ito K, Hirao A, Arai F, Matsuoka S, Takubo K, Hamaguchi I, et al. Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. Nature. 2004;**431**:997-1002

[34] Jang YY, Sharkis SJ. A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche. Blood. 2007;**110**:3056-3063

[35] Huang H, Kim HJ, Chang EJ, Lee ZH, Hwang SJ, Kim HM, et al. IL-17 stimulates the proliferation and differentiation of human mesenchymal stem cells: implications for bone remodeling. Cell Death and Differentiation. 2009;**16**:1332-1343

[36] Sattler M, Winkler T, Verma S, Byrne CH, Shrikhande G, Salgia R, et al. Hematopoietic growth factors signal through the formation of reactive oxygen species. Blood. 1999;**93**:2928-2935

[37] O'Brien JJ, Spinelli SL, Tober J, Blumberg N, Francis CW, Taubman MB, et al. 15-deoxy-delta12,14-PGJ2 enhances platelet production from megakaryocytes. Blood. 2008;**112**:4051-4060

[38] Motohashi H, Kimura M, Fujita R, Inoue A, Pan X, Takayama M, et al. NF-E2 domination over Nrf2 promotes ROS accumulation and megakaryocytic maturation. Blood. 2010;**115**: 677-686

[39] McCrann DJ, Eliades A, Makitalo M, Matsuno K, Ravid K. Differential expression of NADPH oxidases in megakaryocytes and their role in polyploidy. Blood. 2009;**114**:1243-1249

[40] Sardina JL, Lopez-Ruano G, Sanchez-Abarca LI, Perez-Simon JA, Gaztelumendi A, Trigueros C, et al. p22phox-dependent NADPH oxidase activity is required for megakaryocytic differentiation. Cell Death and Differentiation. 2010;**17**:1842-1854

[41] Tang Y, Zhang G, Baird M, Racke F, Zhao W. A novel role of CYP2E1 in human megakaryocyte development. In Vivo. 2014;**28**:1077-1084

[42] Shinohara A, Imai Y, Nakagawa M, Takahashi T, Ichikawa M, Kurokawa M. Intracellular reactive oxygen species mark and influence the megakaryocyte-erythrocyte progenitor fate of common myeloid progenitors. Stem Cells. 2014;**32**:548-557

[43] Yamamoto T, Sakaguchi N, Hachiya M, Nakayama F, Yamakawa M, Akashi M. Role of catalase in monocytic differentiation of U937 cells by TPA: hydrogen peroxide as a second messenger. Leukemia. 2009;**23**:761-769

[44] Del Prete A, Zaccagnino P, Di PM, Saltarella M, Oliveros CC, Nico B, et al. Role of mitochondria and reactive oxygen species in dendritic cell differentiation and functions. Free Radical Biology & Medicine. 2008;**44**:1443-1451

[45] Weitzman SA, Weitberg AB, Clark EP, Stossel TP. Phagocytes as carcinogens: malignant transformation produced by human neutrophils. Science. 1985;**227**:1231-1233

[46] Szatrowski TP, Nathan CF. Production of large amounts of hydrogen peroxide by human tumor cells. Cancer Research. 1991;**51**:794-798

[47] Valgimigli M, Valgimigli L, Trere D, Gaiani S, Pedulli GF, Gramantieri L, et al. Oxidative stress EPR measurement in human liver by radical-probe technique. Correlation with etiology, histology and cell proliferation. Free Radical Research. 2002;**36**:939-948

[48] Liaw KY, Lee PH, Wu FC, Tsai JS, Lin-Shiau SY. Zinc, copper, and superoxide dismutase in hepatocellular carcinoma. The American Journal of Gastroenterology. 1997;**92**:2260-2263

[49] Elchuri S, Oberley TD, Qi W, Eisenstein RS, Jackson RL, Van RH, et al. CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. Oncogene. 2005;**24**:367-380

[50] Van Remmen H, Ikeno Y, Hamilton M, Pahlavani M, Wolf N, Thorpe SR, et al. Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. Physiological Genomics. 2003;**16**:29-37

[51] Galadari S, Rahman A, Pallichankandy S, Thayyullathil F.

Reactive oxygen species and cancer paradox: To promote or to suppress? Free Radical Biology & Medicine. 2017;**104**:144-164

[52] Devi GS, Prasad MH, Saraswathi I, Raghu D, Rao DN, Reddy PP. Free radicals antioxidant enzymes and lipid peroxidation in different types of leukemias. Clinica Chimica Acta. 2000;**293**:53-62

[53] Hole PS, Zabkiewicz J, Munje C, Newton Z, Pearn L, White P, et al. Overproduction of NOX-derived ROS in AML promotes proliferation and is associated with defective oxidative stress signaling. Blood. 2013;**122**:3322-3330

[54] Cooke MS, Evans MD, Dizdaroglu M, Lunec J. Oxidative DNA damage: mechanisms, mutation, and disease. The FASEB Journal. 2003;**17**:1195-1214

[55] Matsui A, Ikeda T, Enomoto K, Hosoda K, Nakashima H, Omae K, et al. Increased formation of oxidative DNA damage, 8-hydroxy-2′-deoxyguanosine, in human breast cancer tissue and its relationship to GSTP1 and COMT genotypes. Cancer Letters. 2000;**151**:87-95

[56] Borrego S, Vazquez A, Dasi F, Cerda C, Iradi A, Tormos C, et al. Oxidative Stress and DNA Damage in Human Gastric Carcinoma: 8-Oxo-7′8-dihydro-2′-deoxyguanosine (8-oxo-dG) as a Possible Tumor Marker. International Journal of Molecular Sciences. 2013;**14**:3467-3486

[57] Gackowski D, Kowalewski J, Siomek A, Olinski R. Oxidative DNA damage and antioxidant vitamin level: comparison among lung cancer patients, healthy smokers and nonsmokers. International Journal of Cancer. 2005;**114**:153-156

[58] Obtulowicz T, Swoboda M, Speina E, Gackowski D, Rozalski R, Siomek A, et al. Oxidative stress and 8-oxoguanine repair are enhanced in colon adenoma and carcinoma patients. Mutagenesis. 2010;**25**:463-471

[59] Zhou FL, Zhang WG, Wei YC, Meng S, Bai GG, Wang BY, et al. Involvement of oxidative stress in the relapse of acute myeloid leukemia. The Journal of Biological Chemistry. 2010;**285**:15010-15015

[60] Gao X, Schottker B. Reduction-oxidation pathways involved in cancer development: a systematic review of literature reviews. Oncotarget. 2017;**8**:51888-51906

[61] Altomare DA, Testa JR. Perturbations of the AKT signaling pathway in human cancer. Oncogene. 2005;**24**:7455-7464

[62] Xu Q, Simpson SE, Scialla TJ, Bagg A, Carroll M. Survival of acute myeloid leukemia cells requires PI3 kinase activation. Blood. 2003;**102**:972-980

[63] Kode A, Mosialou I, Manavalan SJ, Rathinam CV, Friedman RA, Teruya-Feldstein J, et al. FoxO1-dependent induction of acute myeloid leukemia by osteoblasts in mice. Leukemia. 2016;**30**:1-13

[64] Sykes SM, Lane SW, Bullinger L, Kalaitzidis D, Yusuf R, Saez B, et al. AKT/FOXO signaling enforces reversible differentiation blockade in myeloid leukemias. Cell. 2011;**146**:697-708

[65] Jayavelu AK, Moloney JN, Bohmer FD, Cotter TG. NOX-driven ROS formation in cell transformation of FLT3-ITD-positive AML. Experimental Hematology. 2016;**44**:1113-1122

[66] Mikawa T, LLeonart ME, Takaori-Kondo A, Inagaki N, Yokode M, Kondoh H. Dysregulated glycolysis as an oncogenic event. Cellular and Molecular Life Sciences. 2015;**72**:1881-1892

[67] DeBerardinis RJ, Chandel NS. Fundamentals of cancer metabolism. Science Advances. 2016;**2**:e1600200

[68] Weinberg F, Hamanaka R, Wheaton WW, Weinberg S, Joseph J, Lopez M, et al. Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**:8788-8793

[69] Liberti MV, Locasale JW. The Warburg Effect: How Does it Benefit Cancer Cells? Trends in Biochemical Sciences. 2016;**41**:211-218

[70] Han T, Kang D, Ji D, Wang X, Zhan W, Fu M, et al. How does cancer cell metabolism affect tumor migration and invasion? Cell Adhesion & Migration. 2013;**7**:395-403

[71] Cosentino C, Grieco D, Costanzo V. ATM activates the pentose phosphate pathway promoting anti-oxidant defence and DNA repair. The EMBO Journal. 2011;**30**:546-555

[72] Ciccarese F, Ciminale V. Escaping Death: Mitochondrial Redox Homeostasis in Cancer Cells. Frontiers in Oncology. 2017;**7**:117

[73] Yang M, Vousden KH. Serine and one-carbon metabolism in cancer. Nature Reviews. Cancer. 2016;**16**:650-662

[74] Dang L, Jin S, Su SM. IDH mutations in glioma and acute myeloid leukemia. Trends in Molecular Medicine. 2010;**16**:387-397

[75] Zhao S, Lin Y, Xu W, Jiang W, Zha Z, Wang P, et al. Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1alpha. Science. 2009;**324**:261-265

[76] Hagen T. Oxygen versus Reactive Oxygen in the Regulation of HIF-1alpha: The Balance Tips. Biochemistry Research International. 2012;**2012**:436981

[77] Mitsuishi Y, Taguchi K, Kawatani Y, Shibata T, Nukiwa T, Aburatani H, et al. Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. Cancer Cell. 2012;**22**:66-79

[78] Rushworth SA, MacEwan DJ. HO-1 underlies resistance of AML cells to TNF-induced apoptosis. Blood. 2008;**111**:3793-3801

[79] Matoba S, Kang JG, Patino WD, Wragg A, Boehm M, Gavrilova O, et al. p53 regulates mitochondrial respiration. Science. 2006;**312**:1650-1653

[80] Zhao L, Mao Y, Zhao Y, Cao Y, Chen X. Role of multifaceted regulators in cancer glucose metabolism and their clinical significance. Oncotarget. 2016;**7**:31572-31585

[81] Bensaad K, Tsuruta A, Selak MA, Vidal MN, Nakano K, Bartrons R, et al. TIGAR, a p53-inducible regulator of glycolysis and apoptosis. Cell. 2006;**126**:107-120

[82] Prior IA, Lewis PD, Mattos C. A comprehensive survey of Ras mutations in cancer. Cancer Research. 2012;**72**:2457-2467

[83] Efeyan A, Zoncu R, Sabatini DM. Amino acids and mTORC1: from lysosomes to disease. Trends in Molecular Medicine. 2012;**18**:524-533

[84] Li M, Jin R, Wang W, Zhang T, Sang J, Li N, et al. STAT3 regulates glycolysis via targeting hexokinase 2 in hepatocellular carcinoma cells. Oncotarget. 2017;**8**:24777-24784

[85] Khatri S, Yepiskoposyan H, Gallo CA, Tandon P, Plas DR. FOXO3a regulates glycolysis via transcriptional control of tumor suppressor TSC1.

The Journal of Biological Chemistry. 2010;**285**:15960-15965

[86] Kawauchi K, Araki K, Tobiume K, Tanaka N. p53 regulates glucose metabolism through an IKK-NF-kappaB pathway and inhibits cell transformation. Nature Cell Biology. 2008;**10**:611-618

[87] Hsieh AL, Walton ZE, Altman BJ, Stine ZE, Dang CV. MYC and metabolism on the path to cancer. Seminars in Cell & Developmental Biology. 2015;**43**:11-21

[88] Boag JM, Beesley AH, Firth MJ, Freitas JR, Ford J, Hoffmann K, et al. Altered glucose metabolism in childhood pre-B acute lymphoblastic leukaemia. Leukemia. 2006;**20**:1731-1737

[89] Liu T, Kishton RJ, Macintyre AN, Gerriets VA, Xiang H, Liu X, et al. Glucose transporter 1-mediated glucose uptake is limiting for B-cell acute lymphoblastic leukemia anabolic metabolism and resistance to apoptosis. Cell Death & Disease. 2014;**5**:e1470

[90] Song K, Li M, Xu X, Xuan LI, Huang G, Liu Q. Resistance to chemotherapy is associated with altered glucose metabolism in acute myeloid leukemia. Oncology Letters. 2016;**12**:334-342

[91] Chen WL, Wang YY, Zhao A, Xia L, Xie G, Su M, et al. Enhanced Fructose Utilization Mediated by SLC2A5 Is a Unique Metabolic Feature of Acute Myeloid Leukemia with Therapeutic Potential. Cancer Cell. 2016;**30**:779-791

[92] Prata C, Maraldi T, Fiorentini D, Zambonin L, Hakim G, Landi L. Nox-generated ROS modulate glucose uptake in a leukaemic cell line. Free Radical Research. 2008;**42**:405-414

[93] Zhao P, Huang J, Zhang D, Zhang D, Wang F, Qu Y, et al. SLC2A5 overexpression in childhood philadelphia chromosome-positive acute lymphoblastic leukaemia. British Journal of Haematology. 2018;**183**:242-250

[94] You ZM, Zhao L, Xia J, Wei Q, Liu YM, Liu XY, et al. Down-regulation of phosphoglucose isomerase/autocrine motility factor enhances gensenoside Rh2 pharmacological action on leukemia KG1alpha cells. Asian Pacific Journal of Cancer Prevention. 2014;**15**:1099-1104

[95] Ju HQ, Zhan G, Huang A, Sun Y, Wen S, Yang J, et al. ITD mutation in FLT3 tyrosine kinase promotes Warburg effect and renders therapeutic sensitivity to glycolytic inhibition. Leukemia. 2017;**31**:2143-2150

[96] Ryland LK, Doshi UA, Shanmugavelandy SS, Fox TE, Aliaga C, Broeg K, et al. C6-ceramide nanoliposomes target the Warburg effect in chronic lymphocytic leukemia. PLoS One. 2013;**8**:e84648

[97] Shi SY, Lu SY, Sivasubramaniyam T, Revelo XS, Cai EP, Luk CT, et al. DJ-1 links muscle ROS production with metabolic reprogramming and systemic energy homeostasis in mice. Nature Communications. 2015;**6**:7415

[98] Liu CC, Wang H, Wang JH, Wang L, Geng QR, Chen XQ, et al. Serum neuron-specific enolase levels are upregulated in patients with acute lymphoblastic leukemia and are predictive of prognosis. Oncotarget. 2016;**7**:55181-55190

[99] Hitosugi T, Zhou L, Elf S, Fan J, Kang HB, Seo JH, et al. Phosphoglycerate mutase 1 coordinates glycolysis and biosynthesis to promote tumor growth. Cancer Cell. 2012;**22**:585-600

[100] McCleland ML, Adler AS, Deming L, Cosino E, Lee L, Blackwood EM, et al. Lactate dehydrogenase B is required

for the growth of KRAS-dependent lung adenocarcinomas. Clinical Cancer Research. 2013;**19**:773-784

[101] Tahir IM, Iqbal T, Jamil A, Saqib M. Association of BCL-2 with oxidative stress and total antioxidant status in pediatric acute lymphoblastic leukemia. Journal of Biological Regulators and Homeostatic Agents. 2017;**31**:1023-1027

[102] Hu W, Wang X, Yang R. Evaluation of D-dimer and lactate dehydrogenase plasma levels in patients with relapsed acute leukemia. Oncology Letters. 2016;**12**:591-596

[103] Hole PS, Pearn L, Tonks AJ, James PE, Burnett AK, Darley RL, et al. Ras-induced reactive oxygen species promote growth factor-independent proliferation in human CD34+ hematopoietic progenitor cells. Blood. 2010;**115**:1238-1246

[104] Chen WL, Wang JH, Zhao AH, Xu X, Wang YH, Chen TL, et al. A distinct glucose metabolism signature of acute myeloid leukemia with prognostic value. Blood. 2014;**124**:1645-1654

[105] Zhang MY, Liu SL, Huang WL, Tang DB, Zheng WW, Zhou N, et al. Bromodomains and Extra-Terminal (BET) Inhibitor JQ1 Suppresses Proliferation of Acute Lymphocytic Leukemia by Inhibiting c-Myc-Mediated Glycolysis. Medical Science Monitor. 2020;**26**:e923411

[106] Wallace DC. Mitochondria and cancer. Nature Reviews. Cancer. 2012;**12**:685-698

[107] Naoe T, Kiyoi H. Gene mutations of acute myeloid leukemia in the genome era. International Journal of Hematology. 2013;**97**:165-174

[108] Yen K, Travins J, Wang F, David MD, Artin E, Straley K, et al. AG-221, a First-in-Class Therapy Targeting Acute Myeloid Leukemia Harboring Oncogenic IDH2 Mutations. Cancer Discovery. 2017;**7**:478-493

[109] DiNardo C, Botton S, Pollyea D, Stein E, Fathi A, Roboz G, et al. Molecular Profiling and Relationship with Clinical Response in Patients with IDH1 Mutation-Positive Hematologic Malignancies Receiving AG-120, a First-in-Class Potent Inhibitor of Mutant IDH1, in Addition to Data from the Completed Dose Escalation Portion of the Phase 1 Study. Blood. 2015;**126**:1306

[110] Wang Y, Zhang L, Chen WL, Wang JH, Li N, Li JM, et al. Rapid diagnosis and prognosis of de novo acute myeloid leukemia by serum metabonomic analysis. Journal of Proteome Research. 2013;**12**:4393-4401

[111] Schraw JM, Junco JJ, Brown AL, Scheurer ME, Rabin KR, Lupo PJ. Metabolomic profiling identifies pathways associated with minimal residual disease in childhood acute lymphoblastic leukaemia. eBioMedicine. 2019; **48**:49-57

[112] Ye J, Fan J, Venneti S, Wan YW, Pawel BR, Zhang J, et al. Serine catabolism regulates mitochondrial redox control during hypoxia. Cancer Discovery. 2014;**4**:1406-1417

[113] Polet F, Corbet C, Pinto A, Rubio LI, Martherus R, Bol V, et al. Reducing the serine availability complements the inhibition of the glutamine metabolism to block leukemia cell growth. Oncotarget. 2016;**7**:1765-1776

[114] Zaal EA, Wu W, Jansen G, Zweegman S, Cloos J, Berkers CR. Bortezomib resistance in multiple myeloma is associated with increased serine synthesis. Cancer Metab. 2017;**5**:7

[115] Patra KC, Hay N. The pentose phosphate pathway and cancer.

Trends in Biochemical Sciences. 2014;**39**:347-354

[116] Chen Y, Xu Q, Ji D, Wei Y, Chen H, Li T, et al. Inhibition of pentose phosphate pathway suppresses acute myelogenous leukemia. Tumour Biology. 2016;**37**:6027-6034

[117] Elf S, Lin R, Xia S, Pan Y, Shan C, Wu S, et al. Targeting 6-phosphogluconate dehydrogenase in the oxidative PPP sensitizes leukemia cells to antimalarial agent dihydroartemisinin. Oncogene. 2017;**36**:254-262

[118] Poulain L, Sujobert P, Zylbersztejn F, Barreau S, Stuani L, Lambert M, et al. High mTORC1 activity drives glycolysis addiction and sensitivity to G6PD inhibition in acute myeloid leukemia cells. Leukemia. 2017;**31**:2326-2335

[119] Xiao G, Chan LN, Klemm L, Braas D, Chen Z, Geng H, et al. B-Cell-Specific Diversion of Glucose Carbon Utilization Reveals a Unique Vulnerability in B Cell Malignancies. Cell. 2018;**173**:470-484

[120] Starkova J, Hermanova I, Hlozkova K, Hararova A, Trka J. Altered Metabolism of Leukemic Cells: New Therapeutic Opportunity. International Review of Cell and Molecular Biology. 2018;**336**:93-147

[121] Adane B, Ye H, Khan N, Pei S, Minhajuddin M, Stevens BM, et al. The Hematopoietic Oxidase NOX2 Regulates Self-Renewal of Leukemic Stem Cells. Cell Reports. 2019;**27**:238-254

[122] Lee EA, Angka L, Rota SG, Hanlon T, Mitchell A, Hurren R, et al. Targeting Mitochondria with Avocatin B Induces Selective Leukemia Cell Death. Cancer Research. 2015;**75**:2478-2488

[123] Hermanova I, Arruabarrena-Aristorena A, Valis K, Nuskova H, Alberich-Jorda M, Fiser K, et al. Pharmacological inhibition of fatty-acid oxidation synergistically enhances the effect of l-asparaginase in childhood ALL cells. Leukemia. 2016;**30**:209-218

[124] Hole PS, Darley RL, Tonks A. Do reactive oxygen species play a role in myeloid leukemias? Blood. 2011;**117**:5816-5826

[125] McCulloch D, Brown C, Iland H. Retinoic acid and arsenic trioxide in the treatment of acute promyelocytic leukemia: current perspectives. Oncotargets and Therapy. 2017;**10**:1585-1601

[126] Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, et al. Association of reactive oxygen species levels and radioresistance in cancer stem cells. Nature. 2009;**458**:780-783

[127] Kim HM, Haraguchi N, Ishii H, Ohkuma M, Okano M, Mimori K, et al. Increased CD13 expression reduces reactive oxygen species, promoting survival of liver cancer stem cells via an epithelial-mesenchymal transition-like phenomenon. Annals of Surgical Oncology. 2012;**19**(Suppl 3):S539-S548

[128] Lagadinou ED, Sach A, Callahan K, Rossi RM, Neering SJ, Minhajuddin M, et al. BCL-2 inhibition targets oxidative phosphorylation and selectively eradicates quiescent human leukemia stem cells. Cell Stem Cell. 2013;**12**:329-341

[129] Poprac P, Jomova K, Simunkova M, Kollar V, Rhodes CJ, Valko M. Targeting Free Radicals in Oxidative Stress-Related Human Diseases. Trends in Pharmacological Sciences. 2017;**38**:592-607

[130] Yasueda A, Urushima H, Ito T. Efficacy and Interaction of Antioxidant Supplements as Adjuvant Therapy in Cancer Treatment: A Systematic Review. Integrative Cancer Therapies. 2016;**15**:17-39