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Review

Aldehyde Dehydrogenase Enzyme Functions in Acute Leukemia Stem Cells

Garrett M. Dancik[1], Ioannis F. Voutsas[2], Spiros Vlahopoulos[3,*]

[1]Department of Computer Science, Eastern Connecticut State University, Willimantic, CT 06226, USA
[2]Cancer Immunology & Immunotherapy Center, Saint Savas Cancer Hospital, 11522 Athens, Greece
[3]First Department of Pediatrics, National and Kapodistrian University of Athens, 11527 Goudi-Athens, Greece
*Correspondence: sblachop@med.uoa.gr (Spiros Vlahopoulos)

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Abstract

The enzymes that belong to the aldehyde dehydrogenase family are expressed in a variety of cells; yet activity of their main members characterizes stem cells, both normal and malignant. Several members of this family perform critical functions in stem cells, in general, and a few have been shown to have key roles in malignant tumors and their recurrence. In particular, ALDH1A1, which localizes to the cytosol and the nucleus, is an enzyme critical in cancer stem cells. In acute myeloid leukemia (AML), ALDH1A1 protects leukemia-initiating cells from a number of antineoplastic agents, and proves vital for the establishment of human AML xenografts in mice. ALDH2, which is located in mitochondria, has a major role in alcohol metabolism by clearing ethanol-derived acetaldehyde. Haematopoietic stem cells require ALDH2 for protection against acetaldehyde, which can cause damage to DNA, leading to insertions, deletions, chromosomal rearrangements, and translocations. Mutations compromise stem cell function, and thereby threaten blood homeostasis. We review here the potential of targeting the enzymatic activity of aldehyde dehydrogenases in acute leukemia.

Keywords: aldehyde dehydrogenase; drug resistance; immunosuppression; leukemia, myeloid, acute; neoplastic stem cells; gene expression; biomarker

1. Introduction

Aldehyde dehydrogenases are enzymes that oxidize aldehydes to carboxylic acids [1]. Aldehydes are generated during cellular metabolism, and when accumulated they react and form adducts with a variety of biomolecules, causing damage to proteins, DNA, lipids, etc. [2,3].

In the organism, ethanol is metabolized to acetaldehyde that interferes with systems that protect cells from oxidant stress. ALDH proteins oxidize acetaldehyde to acetate, protecting cells and tissues from oxidative damage and helping maintain tissue function. In particular, ALDH proteins protect both normal as well as malignant stem cells (cancer stem cells) from reactive aldehydes and certain cytotoxic drugs such as cyclophosphamide [4,5]. Increases in endogenous formaldehyde deplete blood progenitor cells [6–8]. According to one line of evidence, hematopoietic stem cells require the enzymatic activity of mitochondrial ALDH2 to protect them from the accumulation of acetaldehyde, which causes damage to DNA [3]. Protection of mice or humans from lethal formaldehyde accumulation has been attributed to ALDH2 in combination with cytoplasmic alcohol dehydrogenase 5 (ADH5) [9]. Among other effects, lack of those enzymatic activities (ALDH2 and ADH5) impairs also the differentiation and proliferation capacity of hematopoietic stem cells [10].

Recently, RNA from the gene that encodes ALDH1A1 was shown to associate with disease outcome in acute myeloid leukemia: lower expression of ALDH1A1 distinguished the group of patients with cytogenetics and molecular genetic markers that give them a favorable prognosis [11,12].

2. ALDH in Cancer Cells

Not only normal hematopoietic cells, but malignant cells also gain protection when expressing ALDH2: mesenchymal cells from bone marrow stroma secrete transforming growth factor-β1 (TGF-β1), to induce ALDH2 activity in acute myeloid leukemia (AML) cells via non-canonical TGF-β signaling. Consequently, inhibition of ALDH2 sensitizes AML cells to chemotherapy [13]. The neoplasia that has been most extensively studied in respect to ALDH expression is breast cancer. There, expression of two main isoforms has been linked with malignant progression and ensuing metastasis: ALDH1A3 and ALDH1A1 [14].

When a DNA plasmid was used to express ALDH proteins in breast cancer cell line SUM159, three family members were detected by immunofluerescence in the cytoplasm and in the nucleus (ALDH1A1, ALDH3A1, ALDH7A1), five in the cytosol (ALDH1A2, ALDH1A3, ALDH1L1, ALDH8A1, ALDH9A1), seven in mitochondria (ALDH1B1, ALDH1L2, ALDH2, ALDH4A1, ALDH5A1, ALDH6A1, ALDH18A1), one in peroxisomes (ALDH3A2), one on the cell membrane (ALDH3B1), one

on the cell membrane and in the cytosol (ALDH16A1) and one gave peri-nuclear staining (ALDH3B2) [15]. From all those, at least ALDH1A1 [16] (oral, ovarian, and breast cancer), ALDH1A3 [17] (pancreatic cancer), ALDH2 [18] (liver), ALDH3A1 [19] (melanoma, lung), have been associated with cancer stem cells, and specific phases of malignant progression, including tumor initiation, drug resistance, and metastasis [16]. Isoforms ALDH1L1, and ALDH1L2 in contrast, function in folate metabolism with the former acting as tumor suppressor, while the latter as oncogene [20].

3. ALDH Activity Identifies Progenitor Cell Fractions in Tissues

ALDH activity is mainly measured as fluorescence intensity of a product. Specifically, live cells expressing high ALDH activity are usually identified by the ALDEFLUOR kit and sorted by fluorescence activated cell sorting (FACS) [21]. The principle of this assay is that an uncharged substrate of ALDH is taken up by living cells, where ALDH converts it to a brightly fluorescent, negatively charged product that is trapped in cells that have an intact cellular membrane. The brightly fluorescent ALDH-expressing cells (ALDHbr) are detected in the green fluorescence channel (520–540 nm) of a standard flow cytometer, or can be purified by use of a fluorescence activated cell sorter [22,23].

ALDHbr cells isolated from the human bone marrow, exhibited a higher colony-forming capacity when compared to a cells with low ALDH activity (Capoccia, *et al*. [24]). These cells could function as progenitors for epithelial, endothelial, and mesenchymal lineages [25]. Furthermore, when assayed in a mouse model of myocardial infarction, ALDHbr cells isolated from the human umbilical cord blood had the capacity to augment angiogenesis in the ischemic heart [26]. Lung-resident ALDHbr progenitor cells have shown a higher proliferative and colony-forming potential than cell populations with low ALDH activity, and the capacity to prevent bleomycin-induced pulmonary fibrosis [27]. In general, a variety of tissues harbor ALDHbr cells, which bestow them with a significant regenerative potential. Examples are pancreatic tissue [28], adipose tissue that harbors cells with both adipogenic and osteogenic potential [29], foreskin that harbors cells with multilineage potential [30], skeletal muscle [31], cardiac atrial appendage [32], maternal decidua basalis [33], and trachea [34].

4. ALDH Activity in Acute Leukemia

Several studies have linked increased ALDH activity with acute leukemia stem cells, although the overwhelming majority concerns AML, and some studies have used samples from both acute lymphoblastic leukemia (ALL) and AML.

In the study model of the zebrafish for engrafted human acute leukemia cells, higher ALDH activity, less differentiated cells and a broader and random migration pattern were related with worse clinical outcome after induction chemotherapy for patients [35].

In ALL, and especially in pediatric ALL, ALDH positivity is a marker of immaturity and stemness [36]. From the bone marrow of ALL patients, two cell populations could be separated, normal haematopoietic progenitor cells (ALDH(+)SSC(lo)CD45(hi)Neu5,9Ac2 -GPs(lo)CD34(+)CD38(-)CD90(+)CD117(+)CD133(+)) that differentiated into morphologically discrete, lineage-specific colonies, being essential for autologous HSC transplantation, while leukemic stem cells had the markers (ALDH(+)SSC(lo)CD45(lo)Neu5,9Ac2 -GPs(hi)CD34(+)CD38(+)CD90(-)CD117(-)CD133(-)) [37]. In B-ALL, ALDH+ cells formed 5–7 fold more colonies than ALDHneg cells in methylcellulose [38]. In T-ALL, transcription factor TAL1 activated expression of ALDH1A2, which supported glycolysis and the TCA cycle, NADH production, and ATP production, and decreased the levels of reactive oxygen species *in vitro* and *in vivo*; overexpression of ALDH1A2 accelerated tumor onset and increased tumor penetrance in a zebrafish T-ALL model [39].

In AML, high aldehyde dehydrogenase activity at diagnosis predicts relapse in patients with t(8;21) AML [40]. In general, evidence has been mounting that the protein family of the aldehyde dehydrogenases plays a role in the development of AML; yet the interpretation of their exact biological effect on AML progression remains debatable [41]. Higher ALDH activity was shown in normal hematopoietic stem cells, when compared to AML stem cells in a study of 32 patients and five bone marrow donors [42]. In a larger sample size (n = 104), a minority of patients (24 of 104) had numerous ALDH-positive leukemic stem cells that could not be separated from normal hematopoietic stem cells, were drug-resistant, and had high efficiency of xenograft formation in mice [43]. Nevertheless, cases with increased fractions of ALDH-positive AML cells were shown to derive from immature hematopoietic progenitors, suggesting an explanation for the poor prognosis and therapy resistance of this subgroup, which was attributed to the transmission of stem cell properties [44,45]. It can be therefore hypothesized that ALDH activity is important for AML cells, yet the AML cells that retain ALDH activity are not abundant.

4.1 ALDH Family Members with a Potential Role in Leukemia

Although most, if not all ALDH proteins have been linked to phenomena relevant in malignancy, five members stand out as potential regulators of malignant stem cell activities, which can be implicated in key functions of leukemia stem cells. These are ALDH1A1, ALDH1A3, ALDH2, ALDH3A1, and ALDH3A2.

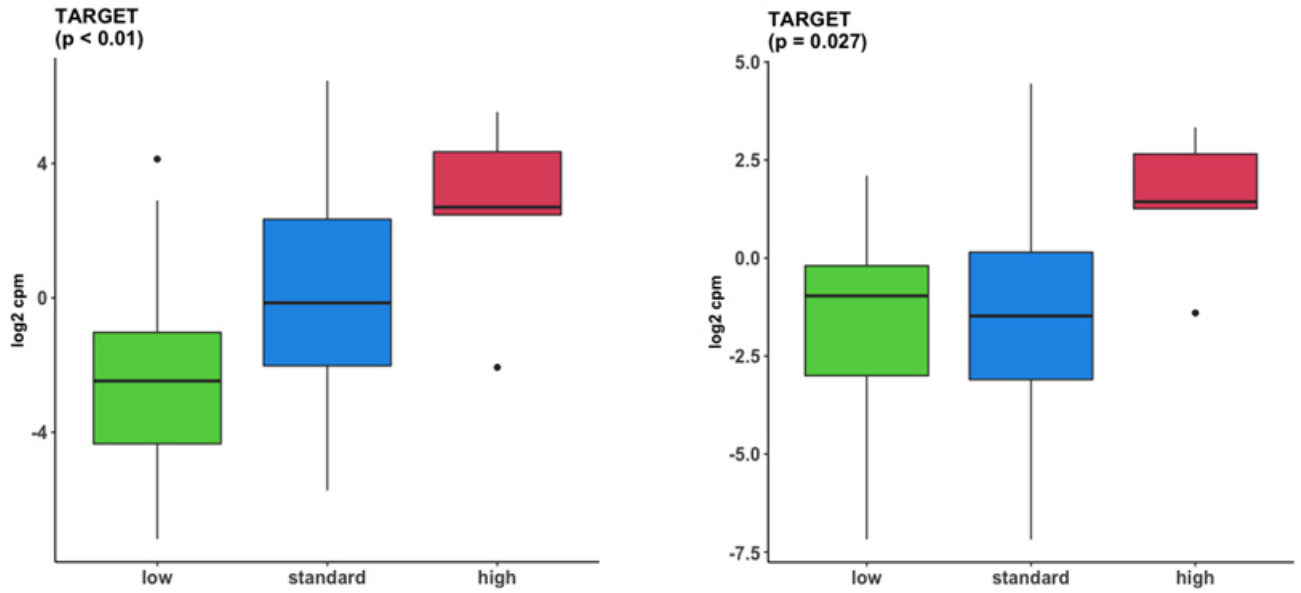

Fig. 1. Comparison of the AML risk group distribution of RNA expression from the genes ALDH1A1 and CALCRL. Association of ALDH1A1 (left) and CALCRL (right) RNA expression (y-axis) with risk groups (x-axis) in the TARGET AML cohort. *p*-values calculated by one-way ANOVA.

4.1.1 ALDH1A1

ALDH family member ALDH1A1, encoded in chromosome 9q21, is located in the cytosol and the nucleus, and catalyzes the second step of the main oxidative pathway of alcohol metabolism.

In general, proteins that belong to the subfamily ALDH1 convert the vitamin A oxidation derivative retinaldehyde, into retinoic acid, which activates retinoic acid receptors (RARs) α, β, and γ. In turn, RARs function as regulators of gene expression that controls differentiation of diverse cell types, and primes tissues for repair, regrowth and regeneration after inflammation or injury, further activating genes of the HOX family; the resulting HOX proteins then induce cellular programs of positional memory during embryogenesis and regeneration [46]. ALDH1A1 may also catalyze the oxidation of toxic lipid aldehydes such as 4-hydroxynonenal and malonaldehyde, and is the enzyme responsible for production of retinoid acid during the late stages of dorsal eye development [47]. In neoplasia, in addition to its known enzymatic activity, ALDH1A1 supports tumor growth via glutathione/dihydrolipoic acid-dependent NAD + reduction [48]. ALDH1A1 overexpression has also been associated with sorafenib resistance in AML and other malignancies [49–52]. ALDH1A1 low expression was recently shown to characterize the favourable risk group in AML [11].

Furthermore, when ALDH1A1 is compared with the recently published strong AML predictor gene CALCRL in the TARGET AML cohort [53], it is found that ALDH1A1 is arguably a better predictor of the favourable risk group (Fig. 1) (For details see **Supplementary Material**).

In physiological function, ALDH1A1 is elevated in hematopoietic stem cells, is a predominant ALDH isoform in mammalian tissues, and the key ALDH isozyme linked to both normal and malignant stem cells [54]. ALDH1A1 in hematopoietic stem cells metabolizes reactive aldehydes and reactive oxygen species, and has a potential role in protection of neurons from 3,4-dihydroxyphenylacetaldehyde [55]. Consequently, decreased expression of ALDH1A1 has been linked to the development of Parkinsons' disease [56]. Furthermore, the ALDH1A1 protein is a critical enzyme for maintaining clarity in human, rat, and mouse lenses, and corneal integrity; ALDH1A1-null mice grow to adulthood, but develop cataracts later in life (by six to nine months of age) [57–60]. Hematopoietic cells from ALDH1A1-deficient mice exhibit increased sensitivity to liver metabolites of cyclophosphamide; however, ALDH1A1 deficiency did not affect the basal function of stem cells from the hematopoietic and nervous system [61].

4.1.2 ALDH1A3

ALDH1A3, encoded in human chromosome 15 (15q26.3), plays a crucial role in the synthesis of retinoic acid in the cytosol, and it is essential for development of the ventral part of the eye [62,63] and cardiomyogenesis [32]. In pancreatic cancer, ALDH1A3 was proposed to accelerate metastasis by augmenting glucose metabolism via increased expression of hexokinase 2 [17]. In other cancer types ALDH1A3 was shown to regulate tumorigenicity and metastasis *in vivo* models, with the net effect depending on the state of the retinoic acid signaling apparatus [14].

4.1.3 ALDH2

ALDH2, encoded in chromosomal locus 12q24.12, has a low Km for acetaldehydes, making mitochondria a subcellular location of efficient acetaldehyde metabolism [64,65].

Analysis of intermediate-risk cytogenetic acute myeloid leukemia patients by RT-PCR has shown that ALDH2 overexpression correlated to shorter overall survival [66]. It has been postulated that ALDH2 expression reflects the adverse prognostic impact of age in AML, while it has been implicated in chemoresistance [67]. AML cells that express low levels of either ALDH1A1 or ALDH2 were sensitive to blocking of the UBE2T/FANCL-mediated ubiquitination pathway for DNA repair; this vulnerability could be ameliorated by expression of recombinant ALDH2, but not ALDH1A1. ALDH2 alone could suppress formaldehyde accumulation, and appeared highly expressed in AML samples carrying TP53 and KMT2A alterations; in contrast, AML samples with low ALDH2 expression were mostly carrying mutations in NPM1, CEBPA, and the RUNX1–RUNX1T1 (AML1–ETO) translocation [68]. Enzymatic activity of ALDH1A generates 9-cis retinoic acid, which is a ligand for retinoic X receptor (RXR), a transcription factor that activates ALDH2 gene expression [69,70]. ALDH1A enzymes also generate all-trans-retinoic acid, which stimulates ALDH2 gene expression via retinoic acid receptors (RARs) [71]. High enzymatic activity of ALDH1A family member proteins can therefore be expected to lead to ALDH2 gene expression.

4.1.4 ALDH3A1

ALDH3A1, encoded in chromosomal locus 17p11.2, is expressed in human cornea and is the main ALDH detected in saliva under physiological conditions; it has a poor affinity for short-chain aliphatic aldehydes, while having a high substrate specificity for medium-chain (6 carbons and more) saturated and unsaturated aldehydes, including 4-hydroxy-2-nonenal, which are generated by the peroxidation of cellular lipids [72–74]. In hepatocellular carcinoma cells, overexpression of ALDH3A1 induced resistance to arsenic trioxide [75].

Also the enzyme ALDH3A2 oxidizes long-chain aliphatic aldehydes to prevent cellular oxidative damage. ALDH3A2 inhibition was synthetically lethal with glutathione peroxidase-4 (GPX4) inhibition; synergistically they triggered ferroptosis in AML cells, which could not be induced by GPX4 inhibition alone [76].

5. AML Features and the Relevance of Leukemia-initiating Cells

AML is the most common form of acute leukemia in adults, commonly accompanied by a poor prognosis, with less than 25% of patients surviving five years after diagnosis [77–79]. AML is a clonal hematopoetic disease that is also associated with a rather high death rate in pediatric (>30%) patients as well. AML is classified according to patient history into primary, de novo AML, which arises in the absence of an identified exposure or prodromal stem cell disorder and non-denovo AML; non-denovo includes secondary AML, representing transformation of an antecedent diagnosis of myelodysplastic syndrome (MDS) or myeloproliferative neoplasm, therapy-related AML developing as a late complication in patients with prior exposure to leukemogenic therapies [80]. From a biological and therapeutic standpoint, relapsed AML is included in non-de novo AML [81].

AML is genetically heterogeneous, yet it involves a rather small number of genetic alterations, with a marked similarity of the molecular spectrum between adult and pediatric patients [82,83]. The World Health Organization defines specific acute myeloid leukemia (AML) disease entities by cytogenetic and molecular genetic subgroups: recurring, balanced cytogenetic abnormalities are recognized in AML, with few exceptions [84].

The clinical course of AML is largely determined by the presence of specific chromosomal aberrations in the cell nucleus, classifying patient cases into low risk, intermediate risk, and high risk. Absence of chromosomal aberrations classifies cases into the intermediate risk group, while cases with detected aberrations are classified into any one of the three categories [85]. In 2017, mutational screening for genes RUNX1, ASXL1, and TP53 was added to the prognostic criteria [86].

Within the heterogeneous population of leukemic cells, researchers identified rarer cells that were rather quiescent, and therefore resistant to antineoplastic treatments that target proliferating cells; these cells could give rise to successful mouse xenografts, their frequency increases after disease relapse and their phenotype has been attributed to epigenetic changes that involve DNA methylation and modification of histones on chromatin, which modulate gene expression, leading to changes in the cellular metabolism, and to resistance to agents that induce stem cell differentiation [87,88].

AML can show durable remission by transplantation of healthy (normal, non-malignant) stem cells; yet substantial work is needed to focus on the effects of therapeutic agents on the interactions between key molecular factors that form the leukemia microenvironment, especially to distinguish between healthy and leukemic stem cells [89]. Research needs to target the leukemia-initiating cells ("leukemia stem cells") selectively, as LSC appear to cause the most severe conditions of AML [90]. A "stemness signature" has been related to risk prediction in 908 patients of diverse AML subtypes, reinforcing this notion [91]. It was then found that approximately 25% of all acute myeloid leukemias expressed low or undetectable levels of ALDH1A1 and that this ALDH1A1-subset of leukemias correlates with good prognosis cytogenetics. ALDH1A1-

cell lines as well as primary leukemia cells were found to be sensitive to treatment with compounds that directly and indirectly generate toxic ALDH substrates including 4-hydroxynonenal and the clinically relevant compounds arsenic trioxide and 4-hydroperoxycyclophosphamide. In contrast, normal hematopoietic stem cells were relatively resistant to these compounds. Using a murine xenotransplant model to emulate a clinical treatment strategy, established ALDH1A1- leukemias were also sensitive to *in vivo* treatment with cyclophosphamide combined with arsenic trioxide [92]. Furthermore, it was found that approximately 25% of all acute myeloid leukemias expressed low or undetectable levels of ALDH1A1 and that this ALDH1A1- subset of leukemias correlates with good prognosis cytogenetics. ALDH1A1- cell lines as well as primary leukemia cells were found to be sensitive to treatment with compounds that directly and indirectly generate toxic ALDH substrates including 4-hydroxynonenal and the clinically relevant compounds arsenic trioxide and 4-hydroperoxycyclophosphamide. In contrast, normal hematopoietic stem cells were relatively resistant to these compounds. Using a murine xenotransplant model to emulate a clinical treatment strategy, established ALDH1A1- leukemias were also sensitive to *in vivo* treatment with cyclophosphamide combined with arsenic trioxide [92]. Subsequently, it was found that low expression of that gene could be statistically associated with the low risk group across multiple cohorts, strongly associating low ALDH1A1 expression with the favorable, low-risk AML group [12].

A hematopoietic stem cell when dividing asymmetrically, it can generate myeloid progenitor cells, which give rise to myeloblasts. Aberrantly developed myeloid progenitor cells or myeloblasts give rise to acute myeloid leukemia [93]. In patients, AML cells with a primitive stem cell phenotype (CD34+/CD38- and high aldehyde dehydrogenase activity) cause significantly lower complete remission rates, as well as poorer event-free and overall survival [94]. One key difference between myelodysplastic syndromes and AML, were balanced cytogenetic rearrangements ($p < 0.0001$), which could thus be associated with initiation of leukaemia [95].

Cytogenetic rearrangements may involve the gene mixed lineage leukaemia (MLL), in which the N-terminal portion of MLL is fused to the C-terminus of the translocation partner; these rearrangements give rise to altered proteins that control epigenetic modifications of nuclear chromatin and regulation of gene expression thus linking cytogenetic rearrangements with molecular changes that show the potential to initiate leukemia [96].

5.1 Maintenance of Defining Features of AML Stem Cells: the Role of Insensitivity to Retinoic Acid

The bone marrow microenvironment of leukemia-initiating cells has a pivotal role in the biology of the disease [97]. Deficient signaling from the vitamin A derivative, all-trans retinoic acid in the hematopoietic microenvironment in mice was shown to cause a myeloproliferative syndrome with significant reduction in the numbers of B lymphocyte subsets and erythrocytes in the bone marrow [98]. In patients with acute promyelocytic leukemia, high blast cell counts and failure to respond to differentiation treatment was associated with low all-trans retinoic acid plasma concentrations, and a high rate of clearance [99]. For the other types of AML, and despite its importance in controlling myeloid differentiation and apoptotic pathways, all-trans retinoic acid has yet to prove itself as a useful agent in the armamentarium of AML therapeutics. The explanation probably lies in the fact that retinoic acid receptor is expressed, but of limited functionality in AML blast cells [100]. However there are both positive and negative associations of CD34+ immature blast cells with sensitivity to all-trans retinoic acid [101,102]. Presence of internal tandem duplications of the FLT3 gene in AML stem cells, reinforces the leukemia-initiating capacity of those cells, which is experimentally demonstrated by successful engraftment into mice [103].

5.2 Pathways Signaling to NFκB Function in AML Stem Cells

A key transcription factor implicated in ALDH1 regulation is nuclear factor kappa B (NFκB) a factor found constitutively active in malignant myeloblast cells of AML [104–106]. Cells normally activate NFκB in response to inflammatory stimuli and disruptions of tissue function [107]. NFκB has an important role in the control of AML stem and progenitor cells, especially in the regulation of interactions between AML cells and their microenvironment [88,108–110]. In poor-prognosis AML, ALDH1A1 RNA is expressed far above the level that is required for the myeloid lineage. Transcription factors such as TLX1/HOX11 and NFκB, both associated with a severe course of AML [111,112] drive ALDH1A1 overexpression, promoting myelopoiesis [113].

To activate ALDH1A1 expression indirectly, NFκB induces expression of micro RNA223-3p, which inhibits expression of ARID1A. ARID1A loss, in turn allows histone acetylation of the ALDH1A1 gene promoter [114]; [115]. Having an established capacity to guide epigenetic changes that guide leukemic stem cell programs, NFκB has been associated with cancer recurrence, and with AML relapse [109,116–118].

In AML, constitutive NFκB DNA-binding activity is frequently mediated by a Ras/PI3-K/PKB-dependent pathway [119]. Also overexpression of FLT3 induces NFκB-dependent transcriptional activity in cultured cells [120]. The constitutive activation of FLT3-ITD also induces NFκB activity [121]. And conversely, FLT3 inhibition or knockdown reduces constitutive NFκB activation in high-risk myelodysplastic syndrome and AML [122].

NFκB is regulated by changes in cellular proteostasis; its native inhibitor, the IkB protein can be degraded by cellular proteolytic systems such as the proteasome or the lysosome, which are mutually regulated through the induction of transcription factors [123,124]. Inhibition of NFκB by the sesquiterpene lactone parthenolide was shown to suppress P-glycoprotein expression and adriamycin resistance in cultured AML stem cells [125,126]. In fact, induction of cell stress combined with NFκB inhibition is a promising approach in AML treatment, due to the connection between proteostatic pathways activated by cell stress and those inducing NFκB activity, as was recently shown in a clinical trial of relapsed patients with acute promyelocytic leukemia [127,128].

One agent that is used in treatment of AML, is the nucleoside cytosine arabinoside [129]. In fact, cytosine arabinoside (cytarabine, ara-C), in high concentrations (>50 uM) inhibits NFκB in some cell types to a certain extent; yet this inhibition is evidently not enough, since after development of drug resistance, ara-C does not suffice as monotherapy for AML [130–132].

On the one hand, mutated, constitutively active FLT3, activates signaling molecules RAS, and PI3K/mTOR which activate NFκB [133]. On the other hand, AML can develop resistance against all inhibitors of FLT3 at some point [134]. Consequently, combination of NFκB and FLT3 inhibitors has been previously suggested by *in vitro* and xenograft studies of AML cells from patients [135,136]. An additional reason to combine inhibitors is the fact that NFκB causes epigenetic changes in nuclear chromatin, which cannot be reversed by inhibition of NFκB itself in the affected cells [137]. Therefore, in spite of its central role in regulation of inflammatory gene expression, NFκB can be difficult to target under certain conditions, due to positive feedback loops that are formed by some of the products of its target genes (Fig. 2), Downstream target genes of NFκB-guided transcription, therefore, such as ALDH1A1, are attractive aims for AML treatment.

6. Inhibition of ALDH as a Potential Therapeutic Approach

ALDH1A1, ALDH1A3, ALDH2 and ALDH3A1 have structural and functional differences in substrate binding site, cofactor dissociation, enzyme kinetics, and rate-limiting steps, which facilitates the design of selective inhibitors and enzymatic activity tracers; in particular, ALDH1A1 substrate binding pocket has a wider access tunnel than ALDH2 or ALDH3A1, allowing ALDH1A1 to accommodate larger and more rigid ligands than other ALDH isoforms [138,139].

ALDH inhibition is not yet established as a therapeutic method in malignant disease; however clinical trials testing ALDH inhibitors in cancer are ongoing. Several trials use disulfiram, an FDA-approved drug to treat alcohol use disorder, to treat central nervous system neoplasms and other types of malignancy [140] (U.S. National Library of Medicine resource platform ClinicalTrials.gov registration numbers NCT01777919, NCT02770378, NCT01907165, NCT03363659, NCT02678975, NCT03151772, NCT03034135, NCT02715609; for an overview see https://clinicaltrials.gov/ct2/results?term=disulfiram&cond=Cancer). Disulfiram inhibits multiple functions of malignant cells, both directly, as well as through its metabolites, especially in the presence of copper that accumulates and is essential for tumor cells [140].

Disulfiram inhibits human lens ALDH1A1 at IC50 values of the micromolar range [59]. In breast cancer cells, ALDH1A1 enzymatic activity facilitated breast tumor growth, and acidified the cytosol to promote phosphorylation of TAK1, activate NFκB signaling, and to increase the secretion of granulocyte macrophage colony-stimulating factor (GM-CSF), which led to myeloid-derived suppressor cell (MDSC) expansion and immunosuppression; disulfiram and chemotherapeutic agent gemcitabine cooperatively inhibited breast tumor growth and tumorigenesis by purging ALDH+ cancer stem cells, and by activating T cell immunity [141].

In fact, in spite of the presence of ALDH activity in hematopoietic stem cells, disulfiram with copper could overcome bortezomib and cytarabine resistance in ALDHbr LSCs via inducing apoptosis and proteasome inhibition [142,143]. Furthemore, another ALDH inhibitor, namely diethylaminobenzaldehyde (DEAB), could induce the expansion of normal human hematopoietic stem cells [144]. DEAB-inhibition of ALDH delayed hematopoietic differentiation and expanded multipotent myeloid cells that accelerated vascular regeneration following intramuscular transplantation into immunodeficient mice with hind-limb ischemia [145]. Yet a further ALDH inhibitor, namely dimethyl ampal thiolester (DIMATE) eradicated leukemia stem cells while sparing normal progenitors, both *in vitro* as well as in mouse xenografts of human AML cells [146].

To date, several inhibitors for ALDH are under development, with the aim a selective effect on specific proteins, especially ALDH1A1. Indeed, novel selective inhibitors for ALDH1A1 were developed recently [147–150]. This field of research shows marked progress due to the fact that ALDH1A1 is implicated in pathological manifestations of inflammatory and metabolic syndromes in addition to cancer [151,152].

Also compounds derived from natural substances exhibit inhibitory activity against ALDH. Notably, the terpenoid citral (3,7-dimethyl-2,6-octadien-1-al), a component of essential oils obtained from several plants, which is used as a food additive and fragrance, inhibits human lens ALDH1A1 at low IC50 values of the micromolar range [59]. Spice and herb extracts have shown a wealth in modulatory activities of ALDH family members ALDH1A1, ALDH1A2, ALDH1A3 and ALDH2; even though it may

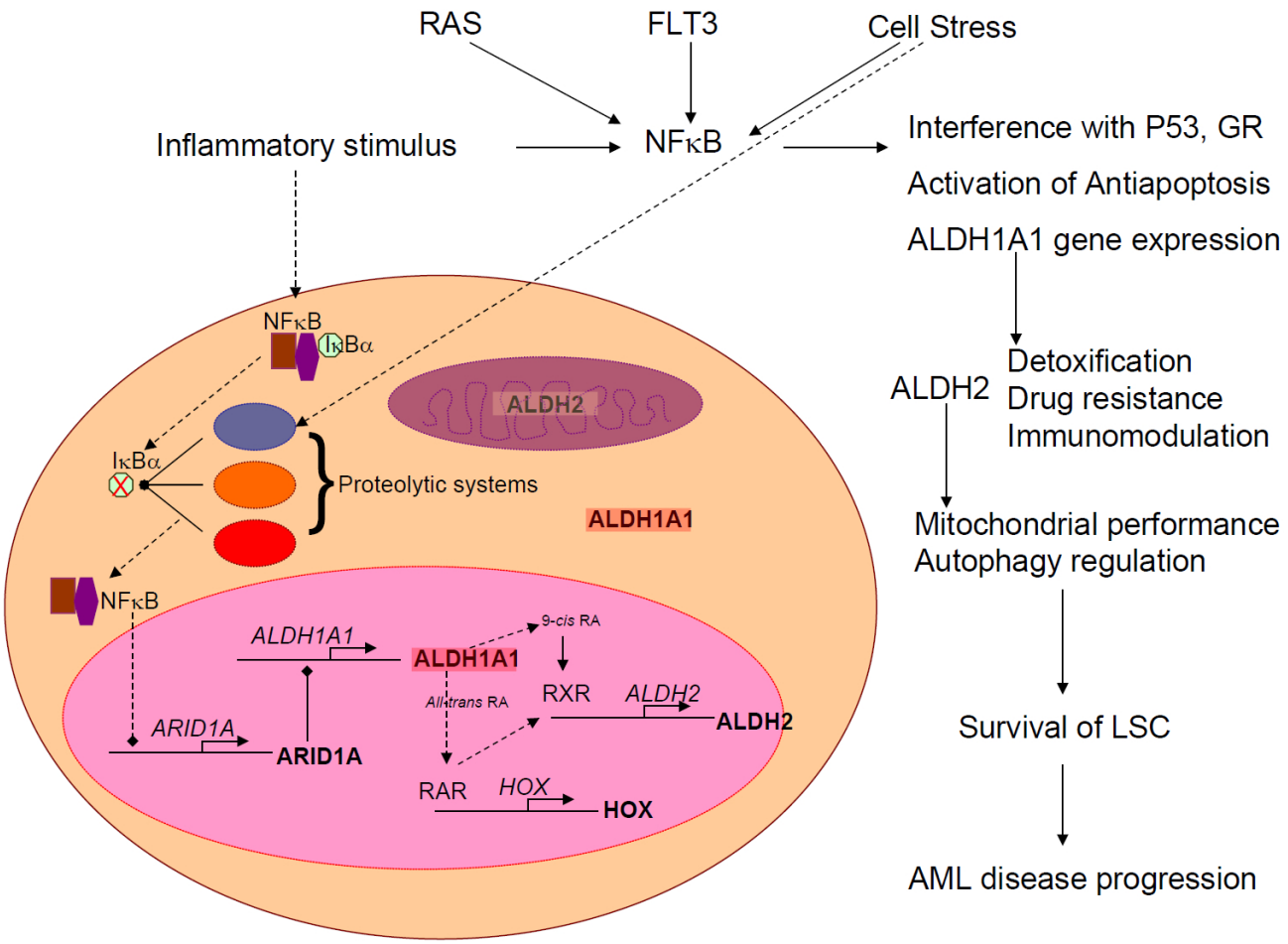

Fig. 2. Overview of the effects of AML stem cell signaling. Due to the extensive phenotypic changes in the cell, upstream signals are rapidly converted to multi-pronged cascades, which become largely self-sustained. Specifically, the induction of expression of diverse downstream genes triggers both developmental changes and alters sensitivity to differentiation signals and to apoptotic stimuli, leading to cells that are substantially unresponsive to physiological homeostatic regulators and at the same time resist pharmacological intervention and escape immune surveillance.

be possible that extracts contain selective modulators, certain extracts manifested general effects, with sage and thyme extracts indicating the potential for complete suppression of the enzymatic activity of all four proteins tested [153].

7. Conclusions

Although initially the principal research finding was that cancer stem cell properties are linked to ALDH1A1, it later became apparent that other family members can be implicated in cancer stem cell function. A wider range of ALDH are linked to cancer stem cell activities than what was expected, and it is likely that ALDH members have the capacity to replace one another under specific conditions, which depends strongly on the host tissue and the metabolic conditions encountered by the malignant cells. This condition is expected to hold in leukemia too, with a particular role of the transitions between bone marrow niche and circulation taking effect in leukemia subtypes that show corresponding transitions between ALDH activity.

Abbreviations

AKT/PKB, RAC-alpha serine/threonine-protein kinase; ALDH, Aldehyde dehydrogenase; AML, Acute myeloid leukemia; ara-c, cytosine arabinoside; CEBPA, CCAAT enhancer binding protein alpha; del, deletion; ELN, European LeukemiaNet; EVI1, ecotropic viral integration site 1; FLT3, Fms-like tyrosine kinase-3; FLT3 ITD, internal tandem duplication of FLT3; inv, inversion; MTOR, kinase, mechanistic/mammalian target of rapamycin; NFκB, nuclear factor kappa B; NOD/SCID, nonobese diabetic/severe combined immunodeficiency mice; NPM1, nucleophosmin 1; OGG1, 8-oxoguanine glycosylase; PD-L1, Programmed death-ligand 1; PI3K, phosphatidylinositol 3-kinase; t, translocation; TLX1/HOX11, T cell leukemia/homeobox 1.

Author contributions

GMD, IFV, SV—conception and design of the research study. GMD—data analysis. GMD, IFV, SV—manuscript writing. GMD, IFV, SV—manuscript editing. GMD, IV, SV—approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at https://www.imrpress.com/journal/FBS/14/1/10.31083/j.fbs1401008.

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