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Aldehyde dehydrogenases: From eye crystallins to metabolic disease and cancer stem cells

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Abstract

The aldehyde dehydrogenase (ALDH) superfamily is composed of nicotinamide adenine dinucleotide (phosphate) (NAD(P)$^+$)-dependent enzymes that catalyze the oxidation of aldehydes to their corresponding carboxylic acids. To date, 24 ALDH gene families have been identified in the eukaryotic genome. In addition to aldehyde metabolizing capacity, ALDHs have additional catalytic (e.g. esterase and reductase) and non-catalytic activities. The latter include functioning as structural elements in the eye (crystallins) and as binding molecules to endobiotics and xenobiotics. Mutations in human ALDH genes and subsequent inborn errors in aldehyde metabolism are the molecular basis of several diseases. Most recently *ALDH* polymorphisms have been associated with gout and osteoporosis. Aldehyde dehydrogenase enzymes also play important roles in embryogenesis and development, neurotransmission, oxidative stress and cancer. This article serves as a comprehensive review of the current state of knowledge regarding the ALDH superfamily and the contribution of ALDHs to various physiological and pathophysiological processes.

Keywords

Aldehyde dehydrogenase; Gene superfamily; Function; Crystallin; Metabolic disease; Stem cell

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1. Aldehyde dehydrogenase superfamily

1.1. Introduction

The ALDH enzymes have usually been defined by their capacity to catalyze $NAD(P)^+$-dependent irreversible oxidation of a wide spectrum of aliphatic and aromatic aldehydes, many of which are generated during the metabolism of endogenous and exogenous compounds (Fig. 1) [1]. The ALDH proteins are found to be present in all subcellular compartments, including cytosol, mitochondria, endoplasmic reticulum and nucleus; several isozymes have been found in more than one of these locations [2,3]. Most of the ALDHs have a wide tissue distribution. Some isozymes display distinct substrate specificity. ALDHs serve to protect cells from the cytotoxic effects of aldehydes by converting them to their respective carboxylic acids [1]. Consequently, they are regarded as being detoxification enzymes. However, some of the carboxylic acids generated by ALDHs function as important molecules in cellular physiology. Examples of these include retinoic acid (RA; essential for development), betaine (osmolyte) and γ-aminobutyric acid (GABA; neurotransmitter).

1.2. Nomenclature

A standardized gene nomenclature system based on divergent evolution was established for the ALDH superfamily at the Ninth International Symposium on Enzymology and Molecular Biology of Carbonyl Metabolism, held June 20–24, 1998, in Varallo Sesia, Italy and it was published in 1999 [4]. Analyses of the sequence of the ALDH isozymes suggest the evolutionary divergence of the ALDH genes from a common ancestor approximately 3 billion years ago [5]. Based on the approved nomenclature system, ALDH proteins belonging to the same ALDH family (e.g. ALDH1 family) are defined as having >40% amino acid identity while two members of the same subfamily (e.g. ALDH1A subfamily) exhibit >60% amino acid identity [4]. A database and website (www.aldh.org) maintained by the laboratory of Dr. Vasilis Vasiliou at the University of Colorado Denver is dedicated to providing a detailed, up-to-date resource for the ALDH gene superfamily [6].

The eukaryotic ALDH gene superfamily currently consists of 24 families that contain putatively functional genes with distinct chromosomal locations. It is anticipated that the number of ALDH families will expand after the inclusion of the bacterial ALDHs (Vasiliou et al., unpublished). Human genes are found in ALDH1–9, ALDH16 and ALDH18 families, which also contain genes from other species including plant and fungi [7].

1.3. ALDH families

The ALDH1A subfamily comprises ALDH1A1, ALDH1A2 and ALDH1A3, all of which synthesize RA from retinaldehyde and, as such, are crucial in regulating RA signaling [4]. These isozymes have a high affinity for the oxidation of both all-*trans*- and 9-*cis*-retinal and exhibit a K_m for these molecules in the low micromolar range [8]. Although ALDH2 officially qualifies as a member of the ALDH1 family, its longstanding name of “ALDH2” (associated with ethanol metabolism) has been grandfathered into the ALDH nomenclature system based on evolutionary divergence. ALDH2 is a mitochondrial enzyme that is primarily involved in the metabolism of acetaldehyde generated during alcohol metabolism

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[1]. ALDH1B1, a mitochondrial enzyme very similar to ALDH2 (75% identical to ALDH2), also contributes to acetaldehyde oxidation, albeit with lower affinity than ALDH2 [9]. ALDH1B1 is a potential colon cancer biomarker [10]. The *ALDH1L1* gene codes for the cytosolic 10-FTHF dehydrogenase (FDH), which converts 10-formyltetrahydrofolate (10-FTHF) to tetrahydrofolate [11]. The *ALDH1L2* gene is very similar to *ALDH1L1* and encodes the mitochondrial FDH that has enzymatic properties similar to its cytosolic counterpart. Unlike ALDH1L1, ALDH1L2 does not metabolize short-chain aldehyde substrates [12]. The ALDH3A subfamily contains the dioxin- inducible ALDH3A1 and ALDH3A2 enzymes, both of which are involved in the oxidation of medium- and long-chain aliphatic and aromatic aldehydes [13,14]. The ALDH3B subfamily consists of two structurally-related genes, *ALDH3B1* and *ALDH3B2*. ALDH3B1 enzyme metabolizes aldehydes generated during lipid peroxidation [15]. As yet, no functional data for *ALDH3B2* gene product exists.

The mitochondrial ALDH4A1, also known as pyrroline-5-carboxylate (P5C) dehydrogenase and glutamate-γ-semialdehyde dehydrogenase, catalyzes the irreversible NAD^+-dependent conversion of P5C to the neurotransmitter, glutamate [16]. The ALDH5A1 isozyme, also known as succinic semialdehyde (SSA) dehydrogenase, catalyzes the NAD^+-dependent conversion of SSA to succinate in the last step GABA catabolism [17]. ALDH6A1 is a mitochondrial enzyme also known as acetyl CoA-dependent methylmalonate semialdehyde (MMS) dehydrogenase. ALDH6A1 is involved in valine and pyrimidine catabolism and catalyzes the oxidative decarboxylation of malonate semialdehyde and MMS to acetyl-CoA and propionyl-CoA, respectively [18]. Human ALDH7A1 has a primary role in the pipecolic acid pathway of lysine catabolism, catalyzing the oxidation of alpha-aminoadipic semialdehyde (AASA) to alpha-aminoadipate [3,19]. ALDH8A1 is a cytosolic enzyme that appears to metabolize retinaldehyde to retinoic acid [20,21]. ALDH9A1 codes for an enzyme that participates in the metabolism of γ-aminobutyraldehyde and aminoaldehydes derived from polyamines [22]. The ALDH16A1 gene encodes an 802-amino acid protein with as-yet unknown function that most likely does not possess catalytic activity (Vasiliou et al., in this volume). ALDH16A1 is a novel and rather unique member of the ALDH superfamily in that it contains two ALDH active site domains (as opposed to one in the other members of the superfamily), four transmembrane domains and a coiled-coil domain. Interestingly, the ALDH16 enzyme active site in frog and many invertebrates (e.g. sea squirt, sea anemone, sea urchin, lancelet, and Trichoplax adhaerens), as well as in bacteria, contains the catalyticallyimportant cysteine residue (Cys-302); in contrast, this residue is absent from the mammalian and fish orthologous protein. The Vasiliou et al. chapter in this issue describes this ALDH as a protein interacting molecule [23].

The ALDH10 family contains plant genes encoding enzymes known as aminoaldehyde dehydrogenases (AMADHs), 4-aminobutyraldehyde dehydrogenases, 4-guanidinobutyraldehyde dehydrogenases, and betaine aldehyde dehydrogenases (BADHs). The ALDH11 family of genes codes for the cytosolic non-phosphorylating glyceraldehyde 3-phosphate dehydrogenases (GAPNs). These enzymes catalyze the irreversible $NADP^+$-dependent oxidation of glyceraldehyde 3-phosphate (GAP) to 3-phosphoglycerate and NADPH [24]. The ALDH12 family contains mostly plant genes encoding -1-pyrroline-5-

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carboxylate dehydrogenases (P5CDH), enzymes involved in the conversion of proline and arginine to glutamate [25]. The ALDH13 family contains a gene found only in Entamoeba histolytica most likely involved in acetaldehyde metabolism [26]. The ALDH14 and ALDH15 families contain yeast and fungi ALDHs [27]. ALDH14 encodes a mitochondrial enzyme that appears to be similar to the microsomal ALDH3A2 [5]. The ALDH15 gene appears to be similar to plant ALDH21 and may play a role in protection against oxidative stress [25]. The ALDH17 gene family comprises genes in Drosophila that code for enzymes with a possible catalytic function as P5CDHs. The ALDH19 family contains plant and bacterial γ-glutamyl phosphate reductases similar to human ALDH18A1. The ALDH21, ALDH22, ALDH23, and ALDH24 families consist of plant genes [25]. No information is currently available regarding the biological role(s) of these enzymes.

2. Other functions of ALDH proteins

2.1. Catalytic functions

Many ALDH proteins possess multiple additional catalytic and non-catalytic functions (Fig. 2). Several ALDHs, including ALDH2, ALDH1A1, ALDH1L1, ALDH1L2 and ALDH9A1, function as esterases [1,28], the catalytic activity of which are physiologically significant in bioactivation of organic nitrates. ALDH2, ALDH1A1 and ALDH1B1 exhibit nitrate reductase activity. In this respect, ALDH2 plays a key role in the bioactivation of therapeutic nitrates, such as nitroglycerin [29].

2.2. Non-catalytic functions

The non-catalytic functions of ALDH proteins range from serving a structural function in the eye to acting as binding proteins in cells. In the eye, several ALDHs have been identified as lens and corneal crystallins. ALDH1 proteins are expressed at very high levels (5–20% of water soluble fraction, which is the definition of crystallin) in the lens of various animal species including human [30–33]. In the cornea, both ALDH1A1 and ALDH3A1 have been identified as corneal crystallins in mammalian species [34,35]. The function of these ALDHs in the ocular tissue may include: (i) contribution to transparency by maximizing light transmission and refraction to the retina [36], and (ii) protection against oxidative damage by absorbing ultraviolet light, metabolizing cytotoxic aldehydes [37], producing NADPH for regeneration of reduced glutathione [38] and physicochemically scavenging hydroxyl radicals [38].

Several ALDHs have been identified as binding proteins for endogenous and exogenous compounds. ALDH1A1 is an androgen-, thyroid hormone- and cholesterol-binding protein [39]. In addition, it interacts with several drugs, specifically quinolone, daunorubicin and flavopiridol [39]. Such interactions may be due to the coiled-coil domains present in the human ALDH1A1 protein at residues 81–99, 114–144 and 172–183, all of which reside within the nucleotide-binding domain. The coiled-coil domain is a highly stable oligomerization motif found in a diversity of proteins that function in gene regulation, cell communication, membrane fusion and drug extrusion [40]. The coil-coiled domain is present in several ALDHs including ALDH3A1 and ALDH16A1. The most intriguing ALDH as a protein-binding molecule appears to be ALDH16A1 (described in detail in this

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issue). As noted above, ALDH16A1 is a novel member of the ALDH superfamily. The species dependence of the critical cysteine residue (Cys-302) in the active site makes it is likely that ALDH16A1 is a binding protein, at least in mammals. In support of this contention, ALDH16A1 has been shown to interact with maspardin, a protein associated with Mast syndrome [41]. In addition, experimental and predictive computational- based molecular modeling evidence from our laboratory suggests that human ALDH16A1 interacts or has the potential to interact with several other proteins associated with uric acid formation, diabetes, vesicular transport and protein degradation. The increasing availability of the crystal structures of ALDHs (Table 1) is facilitating the use of molecular modeling as a tool to explore catalytic and non-catalytic functions of these enzymes.

3. Corneal and lens crystallins

3.1. ALDH1A1, ALDH1A8, ALDH1A9 and ALDH3A1

Some members of the ALDH superfamily have been identified as crystallins in the cornea and lens of both vertebrates and invertebrates [2]. The homodimeric ALDH3A1 is the first enzyme to be categorized as a corneal crystallin [42]. It accumulates in high abundance in the cornea of most mammalian species, representing 5–50% of total soluble proteins depending on the species. Rabbits exceptionally express ALDH1A1, with no detectable amounts of ALDH3A1, in the cornea [34]. Other vertebrates, including chicken, frog and fish, also express ALDH1A1 rather than ALDH3A1 in the cornea [43]. The homotetrameric ALDH1A1 is primarily considered as a lens crystallin in human, where it constitutes 2% of the soluble proteins in lens epithelium [33]. Other members of the ALDH1 family are identified as lens crystallins in the elephant shrew (ALDH1A8/η-crystallin) [44], cephalopods (ALDH1C/Ω-crystallins) [32] and scallop (ALDH1A9/Ω-crystallin) [31].

3.2. ALDH1A1 and ALDH3A1 as protective elements of the eye

Numerous lines of evidence support the notion that ALDH isozymes, being corneal and lens crystallins, confer the necessary protective properties of these ocular tissues. An important protective function for ALDH3A1 and ALDH1A1 in the eye is supported by the observation that spontaneous cataracts develop in *Aldh3a1*$^{-/-}$ knockout and *Aldh1a1*$^{-/-}$/*Aldh3a1*$^{-/-}$ double knockout mice by one month of age, and in *Aldh1a1*$^{-/-}$ knockout mice by 6–9 months [45]. In the same line, these *Aldh*-null mice are highly susceptible to UV-induced cataract formation [45]. Owning to their catalytic and non-catalytic functions, ALDH3A1 and ALDH1A1 play a key role in protecting the eye from ultraviolet radiation (UVR) induced damage (Fig. 3A). First, ALDH3A1 and ALDH1A1 metabolize toxic aldehydes produced by UV-induced lipid peroxidation. Human ALDH3A1 has high affinity for 4-hydroxynonenal (4-HNE), albeit it metabolizes malondialdehyde (MDA) poorly [13]. The presence of ALDH1A1 in the cornea and lens compensates for the absence of ALDH3A1 by oxidizing both 4-HNE and MDA [46]. Second, ALDH3A1 and ALDH1A1 act as antioxidants by serving as direct targets for UV-induced free radicals, thereby providing a passive protective effect to other proteins [47]. Third, NAD(P)H produced during ALDH-mediated metabolism contributes to the antioxidant arsenal of the cornea and lens. NAD(P)H can directly absorb UVR and it is the reducing agent used in the regeneration of antioxidant glutathione [47]. It also helps to maintain a reducing potential for various redox-

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active enzymes, which are involved in protecting eye tissues [48]. Fourth, ALDH3A1 is a major UVR filter. UVR-induced modifications to proteins may lead to enzyme inactivation, partial unfolding and non-native aggregation [49], all of which may be responsible for the accumulation of aggregated proteins in the lens during cataract formation [50]. Studies indicate that direct absorption of UVR energy by ALDH3A1 protects other corneal proteins at the expense of their own molecular inactivation [51].

3.3. ALDH1A1 and ALDH3A1 as structural elements of the eye

Studies using cell culture and experimental animals suggest that ALDH1A1 and ALDH3A1 may function as the structural elements of the cornea, contributing to its transparent and refractive properties (Fig. 3B). In rabbit, the development of postnatal corneal transparency is associated with stromal keratocyte quiescence and increased ALDH1A1 expression; loss of ALDH1A1 expression in the corneal stroma is associated with injury-induced corneal haze [52]. In rat and mouse, ALDH3A1 is undetectable in embryo eyes, but drastically increases in the cornea around 9–14 days after birth, at which time the eye opens [53,54]. In addition, increased light scattering and corneal haze have been observed in ALDH3A1 knockout mice (Vasiliou et al., unpublished), which provide an important piece of evidence supporting a structural role of ALDH3A1 in the cornea *in vivo.* A putative regulatory function of ALDH3A1 on corneal cell proliferation (Fig. 3C) has been proposed based on studies showing (i) a nuclear localization of ALDH3A1 and ALDH1A1 proteins in transfected human corneal epithelial cells [55] and in transfected and normal rabbit corneal keratocytes [2,56], and (ii) an inverse relationship between ALDH3A1 expression and cell proliferation rate in corneal cells *in vitro* [55] and *in vivo* (Koppaka et al., unpublished). Such inhibitory effect of ALDH3A1 on cellular proliferation may have important implications for a regulatory role of ALDH3A1 in the cornea. It may serve as an additional mechanism, by which this crystalline protein participates in maintaining the corneal homeostasis by controlling the mitotic phenotypes of corneal epithelial cells and fibroblasts in response to physiological or stress signals.

4. Metabolic diseases

The importance of ALDH proteins in biological processes is perhaps best exemplified by the associations between *ALDH* gene mutations or polymorphisms and distinct disease phenotypes in humans and rodents. In most instances, the mechanisms by which deficiencies in the ALDH isozymes contribute to the diseases remain to be defined. *Aldh1a1* knockout mice are sensitive to UV light and age-related cataract formation [45]. On the other hand, these same mice are protected from obesity and diabetes [57], possibly due to the recently observed function of ALDH1A1 in regulating gluconeogenesis and lipid metabolism [58] and in thermogenic programming in white adipose tissue [59]. *Aldh1a2* knockout mice are embryonic lethal [60]; mutations in *ALDH1A2* gene are associated with spina bifida [61] and may play a possible causal role in rare cases of human congenital heart disease [62]. *Aldh1a3* knockout mice are also lethal due to defects in nasal development [63]. Mutations in *ALDH1B1* are associated with hypertension and ethanol sensitivity [64,65]; decreased ALDH1B1 expression is found in patients with ethylmalonic encephalopathy, an inherited and severe metabolic disorder [66]. ALDH1B1 has also been proposed as a susceptibility

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locus for mood disorder in the Finnish population [67]. NEUT2 mice, which do not express cytosolic ALDH1L1 due to a micro-deletion within chromosome 6 that results in the loss of *Aldh1l1*, have reduced reproductive efficiency [68,69].

The most common *ALDH* polymorphism, *ALDH2*2*, renders ALDH2 catalytically inactive. By promoting the accumulation of acetaldehyde after ethanol consumption and the consequent unpleasant physiological effects, it is associated with lower consumption of alcohol and a lower incidence of alcoholism. The ALDH2*2 allele has been associated with several pathophysiological conditions related or unrelated to ethanol, including myocardial infarction and hypertension [70,71], increased risk for cancers (reviewed in [1]), liver cirrhosis [72] and late-onset Alzheimer's disease [73].

Aldh3a1 knockout mice are susceptible to cataract formation [45] and mutations in the *ALDH3A2* gene cause Sjögren–Larsson syndrome (SLS) in humans [74]. Mutations in ALDH4A1 gene are associated with type II hyperprolinemia [34], and mutations in ALDH5A1 cause γ-hydroxybutyric aciduria [35]. A deficiency in ALDH6A1 is associated with psychomotor delay and methylmalonic aciduria [75] and a mutation in the *ALDH6A1* gene causes 3-hydroxyisobutyric aciduria [76]. Patients suffering from pyridoxine-dependent seizures have a wide spectrum of mutations in the *ALDH7A1* gene [36]. *ALDH7A1* has also been identified as susceptibility gene for osteoporosis [77] A recent study in 6017 Icelandic subjects identified a rare missense SNP in the ALDH16A1 gene that is associated with gout and elevated serum uric acid levels [78]. Finally, polymorphisms in *ALDH18A1* are associated with hyperammonemia [1].

5. Hematopoietic stem cells

Hematopoietic stem cells (HSCs), rare cells found primarily in bone marrow, generate all hematopoietic lineages, including myeloid cells, lymphocytes, red blood cells and platelets for months to decades [79]. Accordingly, HSCs play a central role in generating and sustaining hematopoiesis for life and are the key cells needed for transfer in blood and marrow transplants used in the treatment of leukemia and other blood diseases. HSCs possess high levels of ALDH catalytic activity [80,81], making them less vulnerable to the cytotoxic effects of the chemotherapeutic agent, cyclophosphamide. Aldefluor®, a fluorescent compound activated by ALDH activity, was found to identify and select for human HSCs as well as mature myeloid progenitors from bone marrow, placenta and peripheral blood and, consequently, has been used as a marker for counting the number of HSCs and myeloid progenitors in these tissues for blood and marrow transplant purposes [76,82–87]. Aldefluor® has also been used to study leukemia and leukemic stem cells (LSCs) and preliminary evidence suggests that levels of activity in these cells may correlate with response to treatment [88–90].

The high level of ALDH activity is suggestive of ALDH serving an important function in HSCs. It is possible that, by regulating intracellular levels of reactive oxygen species (ROS) and/or reactive aldehydes, ALDHs may modulate redox-dependent signal transduction pathways involved in fate decision processes in HSCs [91,92] (Fig. 4). In such a scheme, perturbations in the ALDH-dependent homeostatic control response may lead to the

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accumulation of ROS, reactive aldehydes and other compounds which, at higher (or inappropriate) levels, could foster myelodysplasia and leukemia through protein and DNA damage. Alternatively, ALDH enzymes could modulate HSCs by an effect on retinoic acid metabolism. For this reason, ALDH1A1 has been the focus of most interest in HSCs because of its involvement in retinoic acid metabolism. However, knockout of ALDH1A1 in mice fails to affect HSCs or hematopoiesis, likely due to compensation by other upregulated ALDH isozymes (or related enzymes) [92,93]. Clearly, further research needs to be conducted to elucidate why HSCs possess high ALDH activity and which ALDH isozymes contribute to the activity. The information derived from such studies will provide valuable insights into the involvement of the ALDH isozymes in normal HSC biology and leukemagenesis and potentially identify new therapeutic opportunities. For example, inhibition of ALDH activity may be useful for expanding HSCs, an intervention that could have implications for radioprotection, transplant and regenerative medicine purposes [84,94,95].

6. Cancer stem cells

The milieu of a cancerous tumor consists of heterogenous cell populations. Several recent reports suggest that cancers harbor a small population of cells that possess increased capabilities for self-renewal, tumor initiation, propagation, treatment resistance and disease recurrence. These cells have been termed cancer stem cells (CSCs). CSCs are defined by their functional properties of (i) tumorigenesis (i.e., forming tumors *in vivo*), (ii) self-renewal (i.e., serially transplantable and (iii) differentiation (i.e., generating heterogeneous lineages recapitulating an original tumor) [96]. The CSC hypothesis [97,98] provides a rational basis for understanding some of the complexity of cancer biology (Fig. 5). For example, their existence may explain tumor recurrence after conventional chemotherapy and radiation therapy. In such a scheme, non-CSCs would be targeted by the treatment. The ability of CSCs to remain viable because of their intrinsic survival mechanisms would allow tumor initiation and propagation to be resurrected [99].

High ALDH enzyme activities have been used as a CSC marker for many solid tumors including breast, lung, liver, colon, pancreatic, ovarian, head and neck, prostate [100–113] and melanoma [114,115]. While the Aldefluor® assay can be used to identify CSCs in a freshly dispersed population of cells from a tumor, it cannot be applied for immunohistochemical analyses of formalin-fixed cancer tissues. Given that ALDH1A1 has been considered to be the key isozyme responsible for positive Aldefluor® staining in stem cells [116], tumor specimens have been probed using ALDH1A1 antibodies. Such studies have failed to show a consistent association between ALDH1A1 expression and patient prognosis in several cancers, including breast [108,117–120], ovarian [121,122] and prostate cancers [103,123]. Since the human ALDH superfamily comprises many isozymes [7,27] each often possessing unique tissue distribution, subcellular localization and substrate specificity [1], it is not unreasonable to speculate that the isozyme expressed in each cancer type could differ based on organ- and/or tissue-specificity [1]. Supporting such a speculation are the reports of elevated expression of ALDH1B1 in colon cancer [10], ALDH3B1 in lung, breast, ovarian and colon cancer [124], ALDH3A1 in lung cancer [125], and ALDH7A1 in prostate cancer [103]. Such information will be critical for understanding the

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biological significance of the ALDH isozymes in the tumor cells and, potentially, for facilitating the development of therapies to treat the cancer.

Recently, ALDH1A3 was found to be expressed in Aldefluor®-positive CSCs from human breast cancer [120] and melanoma [115]. As such, it is unlikely that the ALDH enzymatic activity measured by Aldefluor® is due only to ALDH1A1. Furthermore, Marcato and colleagues found that the tumor grade and metastasis correlated with ALDH1A3 expression but not with ALDH1A1 in human breast cancer [120]. This finding underscores the importance of defining the ALDH isozyme responsible for the high ALDH activity in each cancer type. ALDH1A isozymes (including ALDH1A1 and ALDH1A3) oxidize retinaldehyde to RA. RA regulates the expression of a variety of genes through RAR and RXR, nuclear receptors that control the transcription of target genes by interacting with specific DNA sequences known as RA response elements (RAREs) [126]. Located in the promoter region of target genes, RAREs consist of two core hexameric motifs, PuG(G/T)TCA, separated by spacer nucleotides [126,127]. In ALDH-positive CSCs from human melanoma, RA-driven target genes with RAREs and genes associated with stem cell function have been elucidated [115]. In the same study, the ALDH1A isozymes were shown to be essential to the function of CSCs, making ALDH1A isozymes putative therapeutic targets [115]. For example, silencing *ALDH1A* genes (*ALDH1A1* and *ALDH1A3*) decreased cell viability and increased apoptosis in ALDH-positive human melanoma CSCs *in vitro*. Knockdown of ALDH1A reduced tumorigenesis *in vivo*. In addition, ALDH-positive human melanoma CSCs were resistant to chemotherapeutic agents; resistance was attributable to the expression of *ALDH1A* genes. These findings provide compelling evidence in favor of the ALDH isozymes functioning as key molecules governing cell proliferation, survival and chemoresistance of CSCs, i.e., they seem more than simply CSC markers. The capacity of the ALDH isozymes to metabolize cytotoxic aldehydes (arising physiologically or as a result of chemotherapy, radiation or oxidative stress) may also contribute to the survival and drug resistance of CSCs. To prevent cancer recurrence, eradication of CSCs must be an essential part of cancer treatment in addition to eliminating non-stem cancer cells [128]. Given the accumulating and evolving evidence, selective suppression of the ALDH isozymes and genes may be a promising frontier for CSC-directed therapeutics in human cancers. It is hoped that this may spur the development of novel and highly specific inhibitors [129,130].

7. Conclusions

The ALDHs are a family of proteins with diverse biological functions and properties that can (and often do) extend beyond their catalytic activity. Research from the past decade has elucidated potential roles for the ALDH isozymes that would not have been predicted from existing knowledge. The development and availability of isozyme-specific enzyme inhibitors should greatly advance our understanding of the physiological and pathophysiological actions of the ALDHs. Further, they will allow assessment of the relative contribution of catalytic actions versus non-catalytic actions (e.g. protein–protein interactions) to any ALDH isozyme effects. If our past experience with the ALDHs is any guide, we should expect the unexpected from this fascinating protein family.

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Abbreviations

AASA	alpha-aminoadipic semialdehyde
ALDH	aldehyde dehydrogenase
AMADH	aminoaldehyde dehydrogenase
BADH	betaine aldehyde dehydrogenase
CIC	cancer initiating cells
CSC	cancer stem cells
10-FTHF	10-formyltetrahydrofolate
FDH	10-FTHF dehydrogenase
GABA	γ-aminobutyric acid
GAP	glyceraldehyde 3-phosphate
GAPN	GAP dehydrogenase
4-HNE	4-Hydroxynonenal
HSC	hematopoietic stem cell
LSC	leukemic stem cell
MDA	malondialdehyde
MMS	methylmalonate semialdehyde
NAD(P)$^+$	nicotinamide adenine dinucleotide (phosphate)
P5C	pyrroline-5-carboxylate
P5CDH	P5C dehydrogenase
RA	retinoic acid
ROS	reactive oxygen species
SLS	Sjögren–Larsson syndrome
SNP	single nucleotide polymorphism
SP	side population
SSA	succinic semialdehyde
TIC	tumor initiating cells
UVR	ultraviolet radiation

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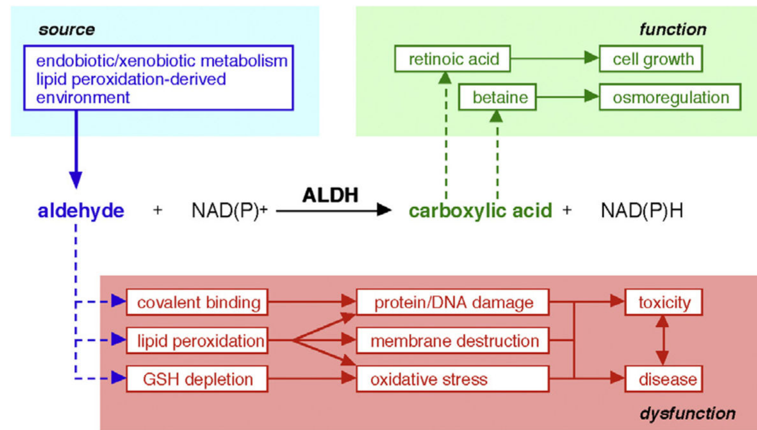

Fig. 1.
Illustration of ALDH catalytic activity.

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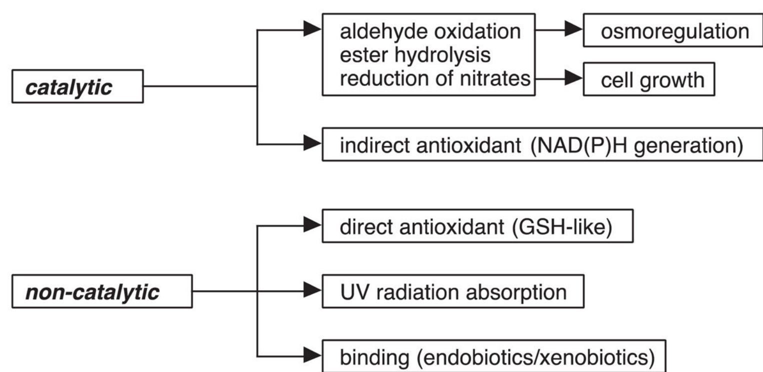

Fig. 2.
Catalytic and non-catalytic functions of ALDH proteins.

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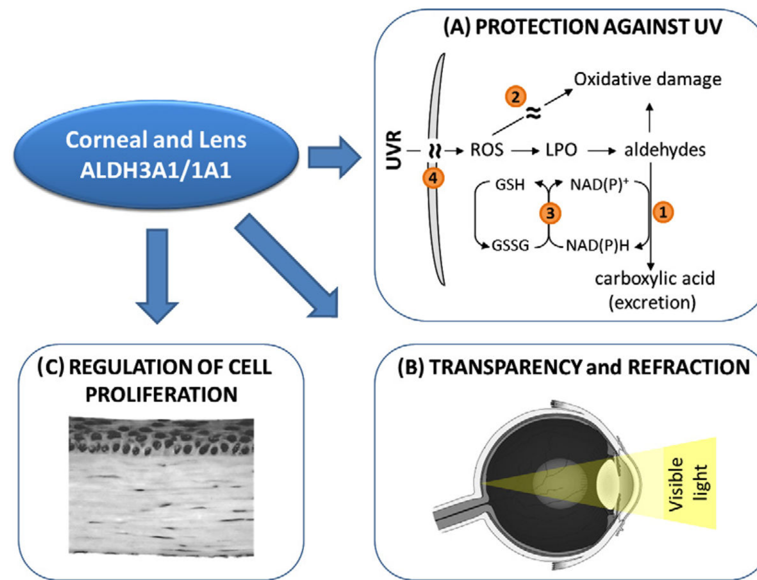

Fig. 3.

Functions of corneal and lens crystallins. (A) Owning to their catalytic and non-catalytic functions, ALDH3A1 and ALDH1A1 proteins protect inner ocular tissues from ultraviolet radiation (UVR) through: ❶ metabolizing toxic aldehydes, ❷ acting as a direct scavengers of reactive oxygen species, ❸ reproducing regenerating antioxidant NADPH and GSH, and ❹ directly absorbing UVR. (B) These corneal crystallins contribute to cellular transparency in corneal stromal keratocytes, supporting a structural role of these ALDH proteins. (C) A putative regulatory function of ALDH3A1 on corneal cell proliferation has also been proposed.

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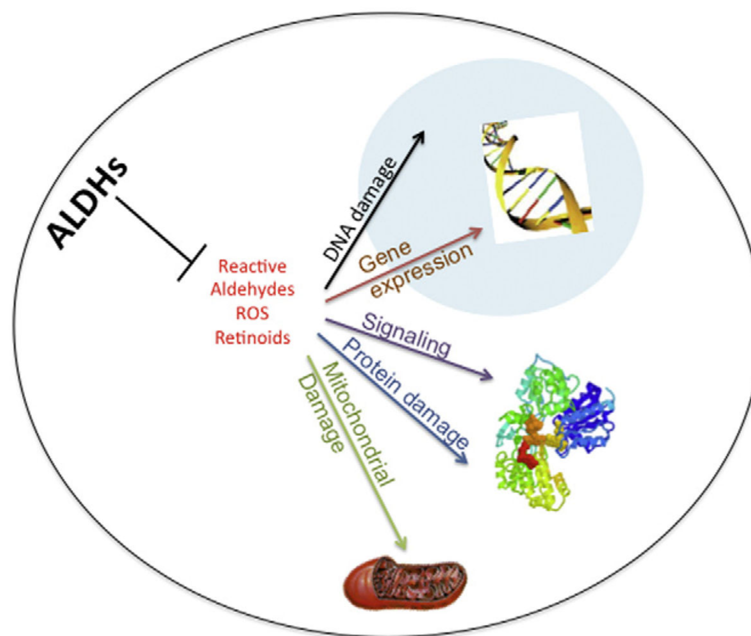

Fig. 4.
Potential mechanisms by which the ALDHs may play a role in normal HSC biology and leukemagenesis. The ALDH proteins as well as their metabolic products including retinoids, ROS, reactive aldehydes and others may directly and indirectly influence a variety of cellular processes including signal transduction, energy metabolism, gene expression and DNA integrity.

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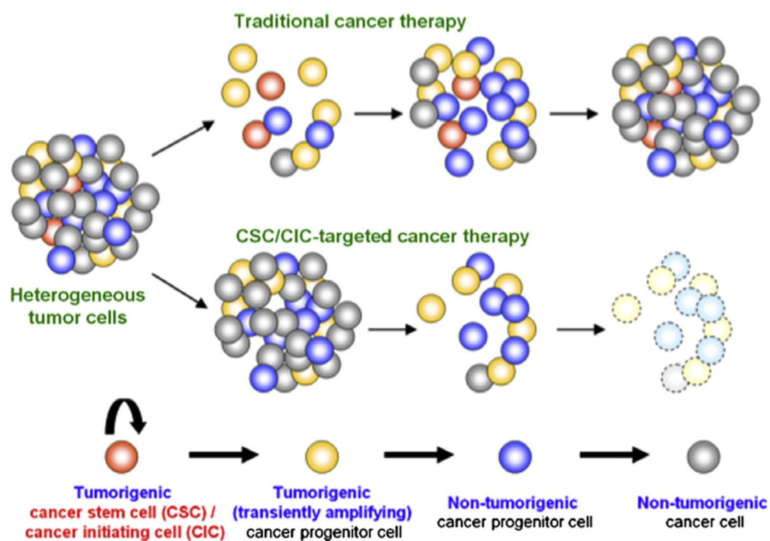

Fig. 5.

Cancer stem cell (CSC) hypothesis. Tumor initiation and growth result from the formation of cancer stem cells (CSCs) or cancer initiating cells (CICs). Tumor recurrence after traditional cancer therapy (such as chemotherapy or radiation) occurs from chemoresistant or radioresistant CSC/CICs. In contrast, CSC/CIC-directed therapeutics targets CSC/CICs and eradicate tumor cells.

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Table 1

Crystal structures of ALDHs.

ALDH enzyme	Species	PDB ID
ALDH1A1	SHEEP	1BXS
ALDH1A2	RAT	1BI9
ALDH1L1	HUMAN	2BW0
ALDH2	HUMAN	3N80
ALDH3A1	HUMAN	3SZA
ALDH4A1	HUMAN	3V9G
ALDH5A1	HUMAN	2W8N
ALDH7A1	HUMAN	2J6L
ALDH10A1	PLANT	4A0M
ALDH18A1	HUMAN	2H5G