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REVIEW

The role of ALDH2 in tumorigenesis and tumor progression: Targeting ALDH2 as a potential cancer treatment

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Abstract A major mitochondrial enzyme for protecting cells from acetaldehyde toxicity is aldehyde dehydrogenase 2 (ALDH2). The correlation between ALDH2 dysfunction and tumorigenesis/growth/metastasis has been widely reported. Either low or high ALDH2 expression contributes to tumor progression and varies among different tumor types. Furthermore, the ALDH2*2 polymorphism (rs671) is the most common single nucleotide polymorphism (SNP) in Asia. Epidemiological studies associate ALDH2*2 with tumorigenesis and progression. This study summarizes the essential functions and potential ALDH2 mechanisms in the occurrence, progression, and treatment of tumors in various types of cancer. Our study indicates that ALDH2 is a potential therapeutic target for cancer therapy.

Abbreviations: 4-HNE, 4-hydroxy-2-nonenal; ALD, alcoholic liver disease; ALDH2, aldehyde dehydrogenase 2; AMPK, AMP-activated protein kinase; BCa, bladder cancer; ccRCC, clear-cell renal cell carcinomas; COUP-TF, chicken ovalbumin upstream promoter-transcription factor; CRC, colorectal cancer; CSCs, cancer stem cells; DFS, disease-free survival; EC, esophageal cancer; FA, Fanconi anemia; FANCD2, Fanconi anemia protein; GCA, gastric cancer; HCC, hepatocellular carcinoma; HDACs, histone deacetylases; HNC, head and neck cancer; HNF-4, hepatocyte nuclear factor 4; HR, homologous recombination; LCSCs, liver cancer stem cells; MDA, malondialdehyde; MDR, multi-drug resistance; MN, micronuclei; NAD, nicotinamide adenine dinucleotide; NCEs, normochromic erythrocytes; NeG, 1,N^2-etheno-dGuo; NER, nucleotide excision repair pathway; NF-κB, nuclear factor-κB; NHEJ, non-homologous end-joining; NRF2, nuclear factor erythroid 2 (NF-E2)-related factor 2; NRRE, nuclear receptor response element; NSCLC, non-small-cell lung; OPC, oropharyngeal cancer; OS, overall survival; OvCa, ovarian cancer; PBMC, peripheral blood mononuclear cell; PC, pancreatic cancer; PdG, N^2-propano-2′-deoxyguanosine; REV1, Y-family DNA polymerase; SCC, squamous cell carcinoma; TGF-β, transforming growth factor β; VHL, von Hippel-Lindau; εPKC, epsilon protein kinase C.

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1. Introduction

ALDH2 belongs to the acetaldehyde dehydrogenase family, comprising of 517 amino acids and four identical subunits that form an autotetraploid to stabilize protein structure. Each subunit consists of three domain structures, including the catalytic domain, the structure of the coenzyme nicotinamide adenine dinucleotide (NAD^+), and the oligomerization domain. Therefore, ALDH2 is a significant participant in the oxidation−reduction reaction of ethanol and endogenous aldehydic products which is set free from lipid peroxidation. The inactive ALDH2 rs671 or low ALDH2 expression causes the accumulation of aldehydic products such as acetaldehyde, 4-hydroxy-2-nonenal (4-HNE), and malondialdehyde (MDA), which are associated with high morbidity of cancer[1,2]. This study reveals that not only is ALDH2 involved in aldehyde metabolism but also plays a key role in the growth of tumors. ALDH2 is mainly located in the mitochondria and cytoplasm. However, ALDH2 was found to contain a substantial amount of exosomes in normal human urine[3]. This is an indication that ALDH2 functions could exceed the ones discussed in this study. ALDH2∗2 heterozygotes or homozygotes with ALDH2 deficiency are associated with a high risk of upper digestive tract cancers among the drinking and smoking populations[4]. Hepatocellular carcinoma (HCC) patients with ALDH2 high expression are associated with a good prognosis[5]. Intriguingly, ALDH2 is a cancer stem cells (CSCs) biomarker and is associated with proliferation, metastasis, and multidrug resistance (MDR) to cancer cell chemotherapy drugs[6]. The role of ALDH2 in tumor tumorigenesis and progression remains unclear, as well as the effect of ALDH2 on the efficacy of tumor therapies. This study outlines the possible role of ALDH2 and its underlying mechanisms in tumorigenesis, tumor progression and cancer treatment in various types of cancer. We present ALDH2 as a potential therapeutic candidate for cancer.

2. The relationship between ALDH2 and cancer

2.1. *ALDH2 polymorphisms is related to cancer occurrence and development*

Substitution of glutamic acid by lysine at 487 codons (Glu487-Lys), also known as rs671 causes the variant ALDH2∗2 allele in exon 12 that affects estimated 560 million East Asians[7]. Heterozygote individual enzyme activity (ALDH2∗1/∗2) was significantly lower (50%) than the wild type, while homozygous mutation enzyme activity (ALDH2∗2/∗2) was between 1% and 4% of the ALDH2∗1/∗1 genotype[8−10]. Several studies have analyzed the relationship between ALDH2 polymorphism and alcohol-related cancers, including head and neck cancer (HNC), esophageal cancer (EC), HCC, colorectal cancer (CRC), gastric cancer (GCA), and breast cancer. Some meta-analyses and major articles reported an increased risk of HNC, EC, HCC, and GCA in patients with the variant ALDH2∗2 allele[11−16]. The occurrence of HNC, ESCC, pancreatic cancer (PC), and bladder cancer (BCa) can result from the combination of ADH1B polymorphisms, the slow/non-functional ALDH2 genotypes, and alcohol[17−21]. In the Japanese population, individuals with a combination of ALDH2∗1/∗2 and ADH2∗2/∗2, little or moderate alcohol consumers, and patients with chronic hepatitis C virus (HCV) infection, are associated with the highest risk of HCC[22,23].

Avincsal et al.[24] reported that, compared with other patients with hypopharyngeal squamous cell carcinoma (SCC), alcoholics with the ALDH2∗2 allele were associated with worse overall survival (OS) and disease-free survival (DFS). In Japanese populations, heterozygous ALDH2 with poor outcomes could be a potential prognostic factor for oropharyngeal cancer (OPC) patients with P16-negative[25]. Analysis of the relationship between the ALDH2 rs671 and HCC postoperative outcome showed that the ALDH2∗1/∗1 genotype had a negative outcome after surgery, whereas HCC patients who carried ALDH2∗2 variants had a better postoperative prognosis[26]. In a recent study, ALDH2 rs671 was associated with a high risk for HCC occurrence in cirrhotic patients with HBV who were heavy drinkers. It was also observed that ALDH2 deficiency in the mouse models of ALDH2 knockout and ALDH2∗1/∗2 knock-in mutant mice aggravated alcohol-associated HCC incidence[27].

ALDH2∗2 variants also had an indirect significant protective effect on the digestive tract cancers for moderate alcohol consumers[11,28]. Liu et al.[29] found that ALDH2 rs671 is significantly associated with low risk of HCC (OR = 0.70, 95% CI = 0.61−0.82). Individuals with ALDH2∗2 variants were less likely to alcohol addicts. A meta-analysis analyzed ALDH2 rs671 polymorphism and CRC risk among 2909 CRC patients and 4903 controls and found ALDH2 polymorphism to be a protective factor of CRC[30]. A recent study showed that there is an indirect relationship between ALDH2 polymorphism and the risk of CRC due to the polymorphism. This polymorphism is thought to reduce the drinking rate[11]. Another study from Japan reported that compared with the ALDH2∗1/∗1 genotype, only the homozygous ALDH2∗2/∗2 genotype was associated with a reduced risk of CRC (OR = 0.55, 95% CI = 0.33−0.93)[28]. In addition, ALDH2 rs671 is a protective factor in ovarian cancer (OvCa), but it is not related to alcohol drinking[31].

The distribution of polymorphisms in ALDH2 among different ethnic populations is different, with an exception in ALDH2 rs671 polymorphism in the Asian population. In the Central European drinking populations, mutation at ALDH2 nucleotide position 248, as well as the ALDH2 +82A > G and ALDH2 −261C > T polymorphisms, are associated with the risk of upper aerodigestive tract cancers[32]. In a European prospective investigation, individuals with both ADH1 (rs1230025) and ALDH2 (rs16941667) variants are likely to develop GCA[33].

There is also a lot of evidence that ALDH2 variants are high-risk factors of ESCC patients who smoke and drink[4,34,35]. Smokers with ALDH2∗2/∗2 genotype are susceptible to lung cancer[36,37]. In conclusion, the variant ALDH2∗2 allele is a genatic risk of smoke and alcohol-induced neoplasm (Table 1[4−6,9,11−47]). These findings suggest that individuals with ALDH2∗2 allele should quit drinking and smoking.

2.2. *Tumor progression associated with ALDH2's alarming expression*

Not only are the ALDH2 variants associated with alcohol-mediated cancer, but ALDH2 expression has also been identified as a possible prognostic marker for several types of cancer. Based on our findings, ALDH2 has numerous effects on tumors. Transcriptional suppression of ADH1A and ALDH2 expression reduced survival and aggravated HCC[44]. To study the lung and liver cancer cell lines, some scientists have used both biochemical and bioinformatics techniques. They found that ALDH2 expression was negatively correlated with DNA base excision repair protein (XRCC1) expression, an indication that low ALDH2 expression was responsible for poor overall survival[48]. Consistent

Table 1 Selected studies showing the relationship of ALDH2 with tumorigenesis and progression.

Tumor type	ALDH2 status	Tumorigenesis	Cancer progression	Ref.
Bladder cancer	Overexpression	–	Unfavorable	6
Esophageal cancer	Mutation	Unfavorable	Unfavorable	4,12,20,34,35,38,39
Head and neck cancer	Mutation	Unfavorable	Unfavorable	11,18,19,24,25,32,40–43
Colorectal cancer	Mutation	Favorable	–	11,28,30
Colorectal cancer	Mutation	Unfavorable	–	16
Gastric cancer	Mutation	Unfavorable	Unfavorable	13–15,33
Hepatocellular cancer	Downexpression	–	Unfavorable	5,9,44
Hepatocellular cancer	Mutation	Unfavorable	Unfavorable	22,23,27
Hepatocellular cancer	Mutation	Favorable	Favorable	26,29
Bladder cancer	Mutation	Unfavorable	Unfavorable	17,45
Pancreatic cancer	Mutation	Unfavorable	–	21
Ovarian cancer	Mutation	Favorable	–	31
Lung cancer	Mutation	Unfavorable	–	36,37
Lung cancer	Activation	–	Favorable	46
Liver cancer stem cells	Overexpression	–	Unfavorable	47

–Not applicable.

with previous studies, downregulated ALDH2 of liver cancer cells caused migration and invasion[5]. One of the mechanisms of ALDH2-modulated HCC progression is that ALDH2 down-expression leads to cell redox status by acetaldehyde accumulation, which then increases the activation of AMP-activated protein kinase (AMPK) pathway in HCC[5]. In addition, a recent study has shown that ALDH2 deficiency induces HCC progression by transmitting extracellular vesicles enriched with oxidized mtDNA from weakened hepatocytes into HCC cells[27]. ALDH2 can therefore be a useful biomarker and target for assessing the prognosis and developing potential HCC therapeutic strategies.

Notably, ALDHs are hallmarks of CSCs which are characterized by cell self-renewal, metastasis, and multidrug resistance to chemotherapeutic drugs. Some studies showed that ALDH2 is not only an enzyme involved in aldehyde detoxification but also a cancer stem marker in tumors. Chen et al.[47] reported that ALDH2 promots the expression of cancer stem biomarkers (*e.g.*, NANOG, OCT4, AND SOX2), leading to the proliferation, migration, and invasion of liver CSCs. ARRB2 (β-arrestin-2) decreased the growth of bladder cancer cells by stem cell marker (CD44, ALDH2, and BMI-1) downregulation[6]. Paradoxically, enhancing the activity of ALDH2 by agonist Alda-1 inhibited the cancer stemness, proliferation, and migration to minimize DNA damage in lung adenocarcinoma cells[46]. However, further research is needed to determine whether ALDH2 is also a cancer stem marker.

3. The regulation of ALDH2

The ALDH2 regulation is summarized in Fig. 1.

3.1. *Transcriptional control of ALDH2*

The major method involved in ALDH2 expression is transcriptional regulation. The crucial role of hepatocyte nuclear factor 4 (HNF-4) in the management of ALDH2 has also been implied in COS-1 and hepatoma cells. Besides, the binding site for HNF-4 has been identified at the ALDH2 promoter domain, which is about 300 bp upstream region from the translation initiation site[49]. Some researchers, however, claim that the nuclear receptor response element (NRRE) is more complicated. The promoter region (at −300−360 bp) of ALDH2 could bind the superfamily of nuclear receptors, comprising the FP330-5 and FP330-3′ elements. The ALDH2 FP330-3′ site serves as a positive transcriptional element that can be activated by retinoid X receptors (RXRs) and HNF-4. However, the present of the family chicken ovalbumin upstream promoter-transcription factor (COUP-TF) and apolipoprotein regulatory protein-1 (ARP-1) can repress the promoter activity of ALDH2 that is activated by RXRs and HNF-4 through binding to the FP330-5′ site[50]. von Hippel-Lindau (VHL) binds to the HNF-4 alpha activating promoter to activate ALDH2 expression, causing the resistance of anthracyclines to clear-cell renal cell carcinomas (ccRCC)[51]. Chen et al.[47] reported that transcription factor FOXM1 binds to the ALDH2 promoter region to promote its expression thereby affecting the expression of NANOG, OCT4, AND SOX2 in LCSCs. Another *in vitro* study showed the ability of vitamin D in mitigating alcoholic liver disease. The mechanism involves vitamin D treatment inducing nuclear factor erythroid 2 (NF-E2)-related factor 2 (NRF2) expression that results in the upregulation of ALDH2 expression to facilitate alcohol metabolism[52]. ALDH2 then mediates ERK phosphorylation through MEK/ERK pathway to increases transcriptional NRF2 through AP-1/TRE[52]. These changes have also been observed in human hepatoma cells[53]. Hyperactivation or MEK/ERK pathway mutations are part of the mechanisms that promote cancer development. It is therefore important to establish the relationships between the MEK/ERK activated pathway and ALDH2 in tumors.

Histone deacetylases (HDACs) are essential epigenetic factors linked to the regulation of non-histone targets. The mTOR signaling in the upstream of HDAC1 is a key mechanism in oncogenesis promoting the survival and proliferation of cancer cell. mTOR/HDAC1 is involved in the regulation of ALDH2 promoter deacetylation, thus, leading to the transcriptional suppression of the ALDH2 gene in HCC[44]. Furthermore, the SET protein (template-activating factor-I b/inhibitor-2 of protein phosphatase-2A) has a role in inhibition of nucleosome acetylation[54]. SET binds specifically to the ALDH2 promoter *via* the SET NAP domain that targets ALDH2's N-terminal histone tails to repress its expression[55].

3.2. *Post-transcriptional control of ALDH2*

Post-transcriptional regulatory factors form RNA expression are microRNAs (miRNAs), whose aberrant expression is associated

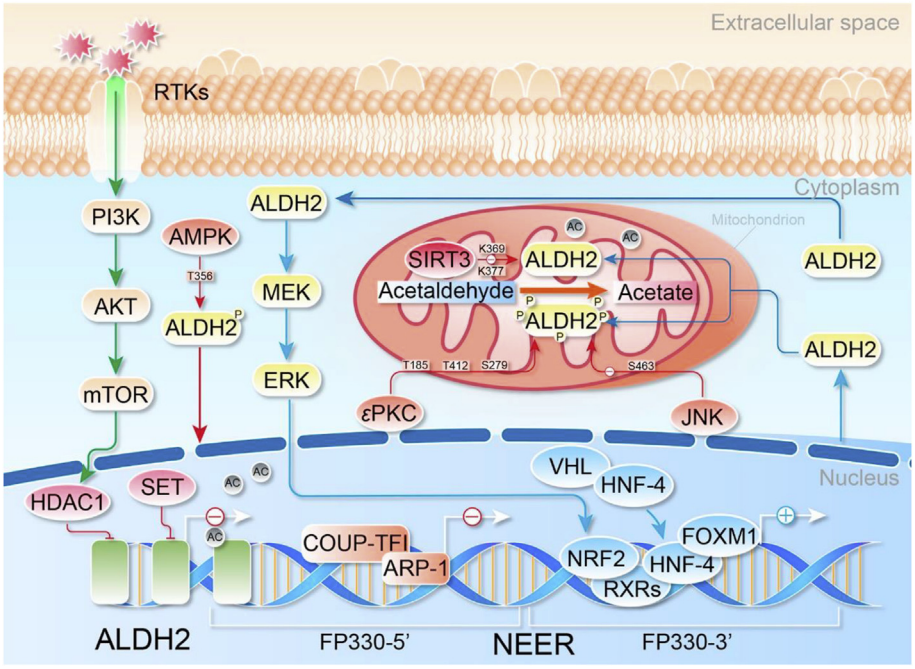

Figure 1 Transcriptional and post-translational level control of ALDH2. PI3K/AKT/mTOR and MEK/ERK pathways are involved in transcriptional control of ALDH2. HDAC1 activated by PI3K/AKT/mTOR/pathway and SET has a role in inhibiting the acetylation of nucleosomes for transcriptional suppression. The activated transcription factors of ALDH2 include NRF2, VHL, HNF-4, RXRs, and FOXM1, which interact with the FP330-3′ element of the promoter region to promote the expression of ALDH2. COUP-TFI and ARP-1 bind to the FP330-5′ element repress the promoter activity of ALDH2. JNK, AMPK, and εPKC can phosphorylate the specific sites of ALDH2 protein for repressing or enhancing its activity. SIRT3 inhibits the enzyme activity of ALDH2 by decreasing its acetylation level. RTKs, receptor tyrosine kinase.

with the development of cancer[56]. MiR-34a is a critical suppressor of tumor progression and metastasis in multiple cancers including HCC[57], CRC[58], triple-negative breast cancer (TNBC)[59,60], and pancreatic cancer[61]. By binding to the 3′UTR of ALDH2, miR-34a downregulates ALDH2 protein and develops its anti-tumor effects by halting the G1 phase thus suppressing migration and invasion of HCC cell[62]. More research should focus on establishing the mechanisms of post-transcriptional regulation of ALDH2.

3.3. *Post-translational regulation of ALDH2*

Epigenetic modification modulates ALDH2 as well. Pyrosequencing-based quantitative analysis was used to detect DNA methylation in GC samples and ALDH2 exhibited significant hypomethylation in gastric tumors[63]. ALDH2 is a target of lysine (Lys) acetylation, and the hyper-acetylation and deacetylation of ALDH2 protein lysine residues alter its biological functions. The major mitochondrial deacetylase sirtuin 3 (SIRT3) plays a crucial role in modulating cellular mitochondrial oxidation reactions. SIRT3 decreased the rate of acetylation of ALDH2 by deacetylation of Lys369 (K369)[64] or Lys377 (K377)[65]. This influenced the enzyme activity to inhibit the normal binding site of the NAD^+ cofactor without affecting the expression of the ALDH2 protein[66]. By modulating cellular metabolism and oxidative stress, deregulated SIRT3 can cause cancer progression[67]. Alcohol influences the activity and/or protein level of SIRT3 in various alcohol-related diseases by increasing ALDH2 activity[68,69]. More researches are needed to ascertain if this mechanism can cause tumorigenesis.

Phosphorylation is an essential post-translational modification of changing protein construction, function, and activity. The activated JNK translocated to mitochondria for phosphorylation the serine 463 (S463) of ALDH2 after carbon tetrachloride (CCl_4) exposure, and attenuated the activity of ALDH2 in rats[70]. Besides, in low-density lipid protein receptor (LDLR) knockout mice, the threonine 356 (T356) of ALDH2 phosphorylated by AMPK could shuttle from cytoplasm into the nuclear thereby suppressing ATP6V0E2 transcription and impairing lysosomal function[71]. Epsilon protein kinase C (εPKC), an oncogenic gene of multiple types of cancer, increases the activity of ALDH2 enzymes by phosphorylation of three ALDH2 sites, including threonine 185 (T185), serine 279 (S279) and threonine 412 (T412)[72].

3.4. *ALDH2 rs671 variants have a dominant-negative effect on protein turnover*

ALDH2 is a tetrameric enzyme, the encode subunit (ALDH2K) of ALDH2*2 gene is lower activity compared with ALDH2*1 transforming the wild-type subunit (ALDH2E)[73]. The ALDH2E protein (half-life >22 h) is more stable than the ALDH2K protein (half-life = 14 h). In addition, the ALDH2K has a dominant-negative effect on enzyme regeneration[73]. The stabilization of ALDH2 in various genotypes is ALDH2*1/*1, ALDH2*1/*2, and ALDH2*2/*273, in descending order. Similarly, Jin et al.[74] suggested that the ALDH2 rs671 variants reduce the level of ALDH2 protein that promotes murine hepatocarcinogenesis in liver cells. However, more research should be done to establish the specific mechanisms of ALDH2 mutant protein decreasing the stability of ALDH2 protein.

4. The related mechanisms of ALDH2 mediated cancers

The related mechanisms of ALDH2 mediated cancers are summarized in graphical abstract.

4.1. ALDH2 and endogenous aldehydes metabolism

ALDH2 is an essential cellular detoxification enzyme for endogenous aldehydes formed in cells by oxidative metabolism. Oxidative reactions promote cancer development due to the overproduction of reactive oxygen species (ROS) and lipid peroxidation. ALDH2 influences the removal of endogenous aldehydes generated by the ROS-mediated peroxidation such as 4-HNE and MDA. MDA levels were found to be significantly higher levels in CRC patients than the healthy controls[75]. CRC production is associated with increased serum MDA levels[76]. Sawczuk et al.[77] stated that MDA also plays a pivotal role in non-invasive diagnosis of BRCA1 mutated breast cancer patients with elevated levels of lipid oxidation products in saliva. In addition, 4-HNE has been reported to be both an endogenous carcinogen and an anti-cancer product. 4-HNE exhibits anti-tumor effects by regulating oncogenic signal pathways and oncogenes downregulation[78]. For example, 4-HNE inhibited nuclear factor-κB (NF-κB) pathway and its anti-apoptotic target BCL-2 leading to cell apoptosis[79]. 4-HNE contributes to colorectal carcinogenesis by activation of the oncogenic transcription factor AP-1 such as c-Jun and NRF2[80]. 4-HNE has also been identified as a major electrophile that binds to DNA and forms a 4-HNE-guanine adduct that induces a high frequency of codon 249 mutation in the *P53* gene[81].

4.2. ALDH2 and the metabolism of exogenous acetaldehyde

ALDH2 plays a key role in acetaldehyde metabolism, which is an intermediate metabolite of ethanol. Individuals with ALDH2 rs671 variants are unable to metabolize exogenous and endogenous aldehydes, leading to the accumulation of acetaldehyde thus forming DNA adducts. The acetaldehyde-derived DNA adducts include N^2-ethylidene-detoxication, *R*- and *S*-a−CH_3−*g*-OH-1,N^2-propano-2′-deoxyguanosine (PdG), and 1,N^2-etheno-dGuo (NeG). Such adducts cause damage to DNA, including DNA mutations, DNA interstrand-cross-links, DNA single-strand breaks, and DNA double-strand breaks[82]. Acetaldehyde causes mutations of DNA by changing GG to TT tandem substitutions in human nucleotide excision repair (NER) deficient cells, and such changes are associated with alcohol-related tumors[83]. Yakawa et al.[84] treated *Aldh2*-knockout mice with ethanol and found that the level of NeG was much higher in the esophagus of *Aldh2*-knockout mice compared to wild-type mice. Matsuda et al.[85] tested 44 blood DNA samples of Japanese alcoholics and detected that the levels of both PdG and NeG adducts were significantly higher in patients with the ALDH2∗1/∗2 genotype compared to alcoholics with the ALDH2∗1/∗1 genotype. All these studies show that acetaldehyde indirectly affects genome stability by creating DNA adducts.

Some studies have shown that human cells with acetaldehyde increase the number of DNA-protein crosslinks (DPCs), leading to genetic and epigenetic instability. The adducts affect genomic stability by slowing the activity of O^6-methylguanine methyltransferase (MGMT) which is a DNA repair protein by protecting DNA from alkylationc[86] and DNA methyltransferase (DNMT) which is a regulator of the epigenetic gene by methylation at the C5 of cytosine[87]. In addition, another potential mechanism that is critical in maintaining genetic and epigenetic stability is the histones modifications. Chen et al.[88] showed that acetaldehyde incubation reduces the level of acetylation for cytosolic histones H3 and H4 N-terminal tails in BEAS-2B cells due to the formation of histone adducts. Acetaldehyde can also form histones H1 adducts and impair its DNA binding functions[89]. Acetaldehyde decreases methylation and acetylation levels thus inducing DPCs to influence the genome function. Further studies are required on DPCs mechanisms and the types of carcinogens they regulate.

4.3. ALDH2 deficiency and DNA damage repair

Cells need to activate and exploit DNA damage repair to protect against acetaldehyde-induced DNA crosslink since ALDH2 deficiency leads to detoxification of acetaldehyde-impaired (Fig. 2). It has been shown that the Fanconi anemia (FA) pathway participates in DNA damage repair. Langevin et al.[90] showed that Fanconi anemia protein (FANCD2) deficient cells were hypersensitive to acetaldehyde exposure; such a phenomenon was also observed in *Fancd2*$^{-/-}$ mice. They further found that the addition of acetaldehyde to cell lines stimulated FANCD2 monoubiquitination, which was necessary to remove DNA interstrand crosslinks. Monoubiquitination of FANCD2 and FANCI form FANCD2−FANCI heterodimers that bind onto DNA and recruit other DNA repair factors to the DNA interstrand crosslinks[91]. As a result, mice with *Aldh2*$^{-/-}$*Fancd2*$^{-/-}$ double-mutant can develop acute leukemia[90]. Moreover, β2-spectrin (β2SP/Sptbn1), a transcriptional cofactor of transforming growth factor β (TGF-β), plays a key role in the repair of alcohol-induced DNA damage by activation of FANCD2 in liver cells[92]. When the TGF-β/β2SP signaling pathway is suppressed, the FA DNA repair pathway could be defective as well. After the absence of the FA DNA repair pathway, non-homologous end-joining (NHEJ) and alternative non-homologous end-joining (Alt-NHEJ) are required to determine the acetaldehyde-induced DNA damage in the mouse hematopoietic system after the failure of the FA DNA repair pathway[93]. The role of the FA pathway cannot be substituted since the NHEJ pathway that induces subsequent mutagenic repair and alternative end-joining. Recent studies have showed that the micronuclei of normochromic erythrocytes (NCEs), one marker of genetic instability, is much more in *Aldh2*$^{-/-}$ *Fancd2*$^{-/-}$ mice (9.5-fold) compared with the wild-type controls. The increase in *Aldh2*$^{-/-}$ mice was 2.9-fold while that of *Fancd2*$^{-/-}$ mice was 1.9-fold[93]. Embryo dysplasia, hematopoietic stem cell dysfunction, and cancer predisposition are aggravated in FA patients diagnosed with ALDH2 deficiency[90,93].

Another way to maintain genome integrity is *via* homologous recombination (HR) repair pathway (Fig. 2). HR family members like BRCA1, BRCA2, and RAD51 have been associated with the FA pathway for DNA crosslink repair[94,95]. Tacconi et al.[95] found that acetaldehyde decreases the growth of BRCA1/2-deficient tumors *in vivo* and using disulfiram, an ALDH2 inhibitor used for clinical treatment of alcoholism also significantly destroys BRCA1/2-deficient cells. Besides, enhanced monoubiquitination of FANCD2 and Ser1524 phosphorylation of BRCA1, the acetaldehyde-induced DNA adducts triggers the FA and BRCA pathways[94]. BRCA1 and BRCA2 variant carriers tend to develop breast and ovarian cancer[96]. In addition to their roles in other cancers, BRCA1 mutation is associated with colorectal cancer[97]. BRCA2 carriers' mutations are likely to cause prostate cancer[98,99]. More researches are needed to establish whether individuals with

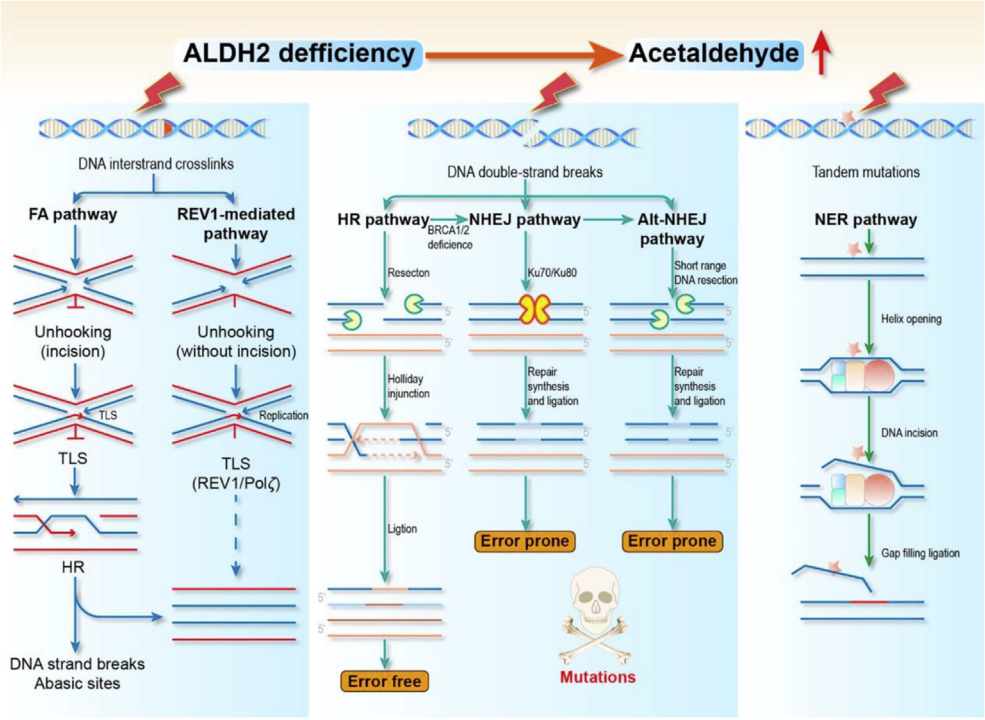

Figure 2 The mechanism of DNA damage repair for ALDH2 deficiency induced DNA damage. The ALDH2 deficiency can lead to acetaldehyde-induced DNA interstrand crosslinks, DNA double-strand breaks (DSB), and tandem mutation, which requires the Fanconi anemia pathway (FA), homologous recombination pathway (HR), Y-family DNA polymerase mediated pathway, classical non-homologous end-joining pathway (NHEJ), alternative non-homologous end-joining repair pathway (Alt-NHEJ), and NER pathway for DNA damage repair. The DNA interstrand crosslinks repair mechanisms involve FA and REV1 pathway. The difference between FA and REV1 pathways is that FA pathway needs to incise for unhooking the crosslink, which leads to DNA strand breaks or abasic sites. However, REV1 repair pathway can operate without excision and promote genome stability. The DSBs repair pathways of acetaldehyde-induced include HR, NHEJ, and Alt-NHEJ. When presents BRCA1/2 mutation or FA pathway inactivation, NHEJ, and Alt-NHEJ pathway are required to repair DSBs and this leads to mutagenesis. NER pathway mediates acetaldehyde-induced tandem mutation repair.

both BRCA1/2 and ALDH2 mutations are more likely to develop cancer.

Recent studies have shown a new way of restoring crosslinks caused by acetaldehyde[100]. The scholars reacted acetaldehyde with guanine to generate PdG which was an acetaldehyde-crosslinked DNA substrate. Two different pathways that repaired these lesions were found. The first was the FA pathway, which determined the DNA interstrand crosslinks caused by acetaldehyde through DNA crosslinks excision[100]. FA pathway increased the frequency of mutations and alerted the mutational spectrum[100]. The second was the Y-family DNA polymerase (REV1) mediated pathway, which was faster with fewer mutations compared with the FA pathway, and involved cutting within the crosslink itself[100].

In addition, acetaldehyde can also react with adjacent guanine, cytosine and adenine, to induce intra-strand crosslinks of DNA (Fig. 2). Matsuda et al.[83] found that acetaldehyde forms intra-strand crosslinks with adjacent guanine bases in human cells thus causing GG to TT tandem mutations. As a result, XP-A protein, a NER pathway related protein, recognizes and binds to the acetaldehyde-mediated DNA damage for repair[83].

4.4. *ALDH2 and autophagy*

Autophagy, a lysosomal degradation system that degrades intracellular components through the lysosome, is used to maintain cellular homeostasis in many human diseases, especially cancer. ALDH2 deficiency induces the accumulation of acetaldehyde and oxidative stress after ethanol treatment. Activated autophagy can serve as a protection against alcohol-related esophageal carcinogenesis at this point[94,101]. However, Guo et al.[102] showed that ethanol and acetaldehyde suppress autophagy in VA-13 cells, although such changes can be reversed by ALDH2. ALDH2 deficiency impairs the beclin-1-dependent autophagy pathway to result in cardiac dysfunction in cardiometabolic diseases[103]. Recent studies have shown that AMPK phosphorylated ALDH2 shuttles to the nucleus to inhibit the lysosomal proton pump protein transcription of ATP6V0E2. This leads to increased foam cells in ALDH2 variant carriers for the attenuated autophagy pathway[71]. To enhance autophagy, ALDH2 enhancement activation is necessary. This is done by attenuating the carbonylation of SIRT1 and stimulating SIRT1 activity, thus improving the deacetylation of nuclear LC3, resulting in increased interaction between LC3 and ATG7 in the cytoplasm[104]. This study shows that in some human diseases, ALDH2 is linked to autophagy regulation, but it is unclear if this association may be the case in cancer. We speculate that autophagy is one of the mechanisms that ALDH2 uses to regulate tumor occurrence and development.

4.5. *ALDH2 and immune system dysfunction*

Seldom studies mention the relationship between ALDH2 and inflammation. Next, we consider describing indirect effects of

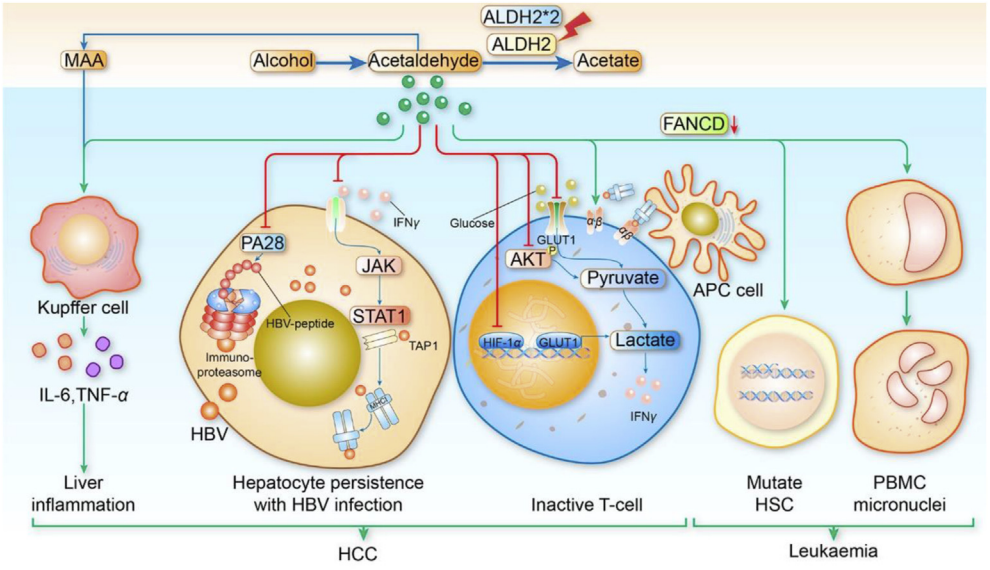

Figure 3 ALDH2 deficiency is related to immune dysfunction and causes HCC and leukemia. The ALDH2 deficiency causes acetaldehyde accumulation which induces liver inflammation, HBV infection persistence, T-cell inactivation, resulting to HCC occurrence. Acetaldehyde accumulation mediates HSC mutation and PBMC micronuclei, and these changes can lead to leukemia.

ALDH2 on the immunity response. ALDH2 regulates the inflammatory response caused by acetaldehyde. Ganesan et al.[105] found that that acetaldehyde decreased the expression of hepatocyte cell surface HBV peptide-MHC class I complexes by decreasing the expression of PA28 and immune proteasome subunits and impairing the signaling pathway of JAK/STAT1/IFNγ. This inhibited CTL activation, leading to persistent HBV infection. In *Aldh2*$^{-/-}$ mice with acetaldehyde exposure, the TCR mutant frequency was much higher compared to *Aldh2*$^{+/+}$ mice after oral administration[106]. *Aldh2* knockout mice fed with chronic ethanol might lead to acetaldehyde accumulation and thereby inhibit T cells activation by suppressing the AKT phosphorylation, the mRNAs expression of glucose transporter 1 (*Glut1*) and hypoxia-inducible factor-1α (*Hif-1α*), both of them mediate aerobic glycolysis[107]. In addition, in *Aldh2*$^{-/-}$ *Fancd2*$^{-/-}$ mice, deletions and rearrangements of hematopoietic stem cell by alcohol and endogenous aldehydes resulting from DNA damage were observed[93], which could destroy the standard immune system. ALDH2 deficient alcohol consumers have an increased frequency of micronuclei[108,109], leading to another lymphocyte chromatid exchanges[110]. Besides, acetaldehyde can combine with MDA generated by lipid peroxidation of membranes as MAA which modifies liver cytosols as the immunogen can contribute to autoimmune hepatitis in patients who take alcohol[111]. MAA adducts modulated immune response has also been associated with the pathogenesis of liver injury with alcohol exposure[112,113]. MDA adducts promote peripheral blood mononuclear cell T-cell proliferation in patients with ALD. This stimulates cellular immune responses that result into advanced ALD[114]. Therefore, ALDH2 indirectly regulates the immune system due to its role in aldehydes metabolism and acetaldehyde adducts. Our previous research showed that *Aldh2* down-regulation increases CD3$^+$ and CD8$^+$ T lymphocyte tumor infiltration, and significantly suppresses tumor growth and progression in our murine CRC model (unpublished data). The ALDH2-mediated immune system dysfunction could be one of the mechanisms leading to cancer (Fig. 3).

5. ALDH2 and cancer therapy

5.1. *Chemotherapy*

Chemotherapy is a significant cancer treatment method. However, MDR is still a problem in chemotherapy. Gemcitabine is a chemotherapeutic antimetabolic agent for non-small-cell lung (NSCLC), gastrointestinal and genitourinary cancer ALDH2 is a cancer stem cell marker for bladder cancer as the downregulation of ALDH2 can make bladder cancer cells gentamicin-sensitive, both *in vitro* and *in vivo*[6]. Mitoxantrone (MTX) is an antimetabolic drug used to treat many types of cancer, including breast cancer, leukemia, lymphomas, etc. MTX toxicity is associated with ALDH2 expression, particularly with ALDH2 variants[115].

While microtubule inhibitors induce drug resistance acquisition in cancer therapy, Wang et al.[116] found that ALDH2 silently increases the cytotoxicity of A549/Taxol and KB/VCR cells to Taxol or vincristine (VCR), respectively, by reducing the effects of cancer stem cells. Disulfiram (DSF), a small molecule-based drug used for the treatment of alcohol addiction targeting ALDH2, irreversibly inhibits the activity of ALDH2's catalytic Cys302 through its metabolic agents, such as *S*-methyl-*N*,*N*-diethyldithiocarbamate (DETC) and *S*-methyl-*N*,*N*-diethyldithiocarbamate (Me-DDTC) that are converted by hepatic thiol methyltransferases[117]. DSF attenuates ALDH2 activity *in vivo* but not *in vitro*. DSF induces apoptosis of multiple cancer cells when it reacts with copper ions to form copper diethyldithiocarbamate ($Cu(DDC)_2$), a major active ingredient[116]. Skrott et al.[118,119] suggested that disulfiram kills cancer cells by suppressing P97/NPL4 that is essential for the turnover of proteins involved in stress-response pathways, rather than inhibiting ALDH activity. In addition, DSF cannot specifically inhibit the activity of ALDH2 without effecting other ALDH isozymes like ALDH1 which is the marker of CSCs in many cancers. The main mechanisms of DSF reversing microtubule inhibitor resistance in targeting ALDH2 need more research. Many clinical trials have considered

Table 2 Clinical trials of disulfiram combined chemotherapy.

Tumor type	Phase	Treatment	Clinicaltrials.gov identifier
Breast cancer	II	Disulfiram, vinorelbine Cisplatin Copper	NCT04265274
Pancreatic cancer	II	Nab-paclitaxel/Gemcitabine/FOLFIRINOX + Disulfiram/copper	NCT03714555
Non-small cell lung cancer	II	Chemotherapy (cisplatin, novelbine) +/− Disulfiram	NCT00312819
Pancreatic carcinoma	I	Gemcitabine hydrochloride, Disulfiram	NCT02671890

incorporating the use of DSF with chemotherapy for cancer treatment (Table 2).

VHL-deficient ccRCC cells present enhanced sensitivity to anthracyclines, and ALDH2 is transcriptionally downregulated by VHL deficiency, which then helps to mitigate anthracycline resistance in ccRCC cells[51]. The main toxic side effect of anthracycline treatment in cancer patients is cardiotoxicity. Doxorubicin is one of the anthracyclines that affects cardiac geometry and functions by impairing mitochondrial integrity and inducing cardiac functional defect. However, ALDH2 overexpression or the ALDH2 agonist Alda-1 can relieve these effects, which mediated TRPV1-related mitochondrial integrity protection pathway[120]. Sun et al.[121] reported that upregulation of 4-HNE and PI3K/AKT mediated autophagy is another mechanism of doxorubicin-induced cardiac defect. However, ALDH2 upregulation or Alda-1-offered protective action can reverse these effects[121].

ALDH2 is associated with the detoxification of reactive aldehydes, including 4-HNE, MDA, and acrolein, generated from ROS-mediated lipid peroxidation. Cisplatin treatment produces a large amount of ROS. Some scholars found that mice with an ALDH2*2 variant were sensitive to cisplatin for increasing ROS levels[122]. Moreover, cells with SET overexpression (HEK293/SET) are more likely to be destroyed by cisplatin compared with HEK293 cells, which is partly caused by low ALDH2 expression and high DNA damage response[55].

5.2. *Molecular targeted treatment*

Molecular targeted inhibitors that block the specific molecules have been a breakthrough in cancer treatment. Trastuzumab/pertuzumab antibodies have been approved for the treatment of human epidermal growth factor receptor 2 (HER2) positive breast and gastric cancer. Komarova et al.[123] reported that trastuzumab and pertuzumab plant biosimilars cooperated with DSF are more effective in killing breast cancer cells compared with pertuzumab plant biosimilars treatment alone. In another study, DSF inhibited the growth of BRCA1/2-deficient patient-derived tumor xenograft cells (PDTCs) that was resisted to treatment of poly(ADP-ribose) polymerase (PARP) inhibitors therapy[95]. More research should focus on establishing whether targeting ALDH2 is the main mechanism used by DSF in enhancing molecular targeted therapy.

5.3. *Immunotherapy*

There are several approaches to cancer immunotherapy, including active vaccination, chimeric antigen receptor T cell therapy, and immune checkpoint blockade. Intravesical Bacillus Calmette-Guerin is an effective treatment in urothelial precancerous lesions. The ALDH2 expression level was lower in the Bacillus Calmette-Guerin-treated group compared with the non-treated group[124]. Some researchers used proteomic profiling of clinical melanoma patients undergoing anti-programmed death 1 (PD-1) immunotherapy to determine the prognostic biomarkers of immunotherapy response[125]. They found that mitochondrial metabolic pathways including branched-chain amino acid degradation, fatty acid oxidation, and oxidative phosphorylation activation are related to high response to immunotherapy[125]. ALDH2 is one of the proteins in ranched-chain amino acid degradation, whose expression is higher in the anti-PD1 responder group compared to the patients of the non-response patients group[125]. Our study also shows that a combination of ALDH2 inhibition and PD-1 blockade, which increases tumor infiltration of TILs and prevents immune evasion, is a novel strategy for potentiating immunotherapy in murine CT26 colon cancer model (unpublished data).

6. Conclusions and perspectives

ALDH2 plays a key role in ethanol metabolism. This study, however, shows that ALDH2 is correlated with occurrence and progression of cancer. While genetic and epigenetic regulation of ALDH2 expression and the function of ALDH2 in the development of cancer has attracted a lot of research, there remains a lot of gaps in the subject. For instance, the role of ALDH2 in oncogenic signaling pathways is only partially understood. A better understanding of this can help in understanding the functions of ALDH2 in cancer. ALDH2 also exists in different parts of cells like mitochondrion, cytosol, nuclear, etc. Exosomal ALDH2 was found in normal human urine, and we suspect that cancer cells can secrete ALDH2. In addition, the dominant participant in aldehyde metabolism is ALDH2 in mitochondria, whereas the functions of ALDH2 in other locations are still unknown.

ALDH2 has a double-edged activity. It can be antioncogenic by reducing the damaging effects of aldehydes, and be oncogenic as well by promoting drug resistance and signaling cell survival. ALDH2*2 affects about 30%−40% Asians and about 8% global populations. ALDH2*2 variant carriers are susceptible to HNC, EC, GCA and HCC due to acetaldehyde accumulation. However, the correlation between CRC and ALDH2*2 remains confusion. Regarding the protection of cancer, if these individuals have no other alternative, they could use the ALDH2 activator to prevent cancer, especially populations with both ALDH2 and FANCD or BRCA mutations. Some studies suggest that ALDH2 overexpression or high activity is correlated with MDR, such as antimetabolic medicines, microtubule inhibitors, and anthracyclines. Accordingly, perhaps individual-based therapy is necessary

for ALDH2 variant carriers, and targeting it could be a novel method to overcome MDR in patients with ALDH2 overexpression. Our study also indicates that downregulation of ALDH2 could enhance PD-1 blockade immunotherapy in murine CT26 colon cancer model (unpublished data). In addition, the ALDH2 inhibitors are designed to only target their activation sites for suppression without affecting their expression. More researches should be done to establish whether the ALDH2 expression inhibitors are superior to their activity antagonists in cancer treatment. The study shows that ALDH2 regulates the proliferation, invasion, and migration of cancer cells. More researches are required to establish whether ALDH2 can serve as a cancer stem cell marker. More researches should also be done to establish whether ALDH2 expression level is associated with the effect of immunotherapy in solid tumors.

In summary, many researchers have elaborated the potential functions of ALDH2 in cancer treatment. Populations with ALDH2*2 polymorphism are susceptible to cancers such as EC, GCA, and HCC. In addition, ALDH2 is also associated with cancer prognosis because it can be a cancer stem cell marker that regulates the proliferation, invasion, migration, and MDR of cancer cells. Many theories about cancer occurrence and development caused by ALDH2 deficiency include: gene mutations, epigenetic instability, autophagy, and immune system dysfunction. Meanwhile, the regulation of ALDH2 expressions such as transcriptional factors, post-transcriptional factors, and post-transcriptional modulators was obtained and identified. ALDH2 expression is associated with the therapeutic effect of chemotherapy, molecular targeted treatment, and immunotherapy. A better understanding of the relationship between ALDH2 and cancer can provide a basis for clinical use, and new strategies of treating cancer patients by targeting ALDH2.

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Author contributions

Hong Zhang wrote the paper. Liwu Fu revised the manuscript.

Conflict of interest

The authors declared no conflict of interest.

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