Title Page

Research progress on the correlation between acetaldehyde dehydrogenase 2 and hepatocellular carcinoma development

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**Running Title Page**

**Running title:** Research progress of ALDH2 and HCC

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Abstract
Hepatocellular carcinoma (HCC) is the predominant pathological type of primary liver cancer. It is a malignant tumor of liver epithelial cells. There are many ways to treat HCC, but the survival rate for HCC patients remains low. Therefore, understanding the underlying mechanisms by which HCC occurs and develops is critical to explore new therapeutic targets. Aldehyde dehydrogenase 2 (ALDH2) is an important player in the redox reaction of ethanol with endogenous aldehyde products released by lipid peroxidation. Increasing evidence suggests that ALDH2 is a crucial regulator of human tumor development, including HCC. Therefore, clarifying the relationship between ALDH2 and HCC is helpful for formulating rational treatment strategies. This review highlighted the regulatory roles of ALDH2 in the development of HCC, elucidated the multiple potential mechanisms by which ALDH2 regulates the development of HCC, and summarized the progress of research on ALDH2 gene polymorphisms and HCC susceptibility. Meanwhile, we envisioned viable strategies for targeting ALDH2 in the treatment of HCC.
Significance statement: Numerous studies have aimed to explore novel therapeutic targets for HCC, and ALDH2 has been reported to be a critical regulator of HCC progression. This review discusses the functions, molecular mechanisms, and clinical significance of ALDH2 in the development of HCC, and examines the prospects of ALDH2-based therapy for HCC.

Keywords: hepatocellular carcinoma, acetaldehyde dehydrogenase 2, susceptibility, gene polymorphism

1. Introduction

Liver cancer is the sixth most prevalent cancer and the third leading cause of cancer-related mortality worldwide (Singal et al., 2023). A global cancer statistics report estimated that approximately 905,700 people were diagnosed with liver cancer in 2020, and 830,200 patients died of the disease (Sung et al., 2021).

Hepatocellular carcinoma (HCC) is one of the main subtypes of liver cancer, and the etiology and pathogenesis of HCC have been the focus of much academic attention. hepatitis B virus (HBV) and hepatitis B virus (HCV) are the most important etiological factors of HCC and accounts for 80% of HCC cases globally (Yang et al., 2017). The etiology of HCC has gradually shifted from viral to non-viral factors with the promotion of anti-HBV drugs, an increase in HBV vaccination strategies, and the discovery of direct antiviral drugs (Akinyemiju et al., 2017). The primary treatment strategies for HCC presently include hepatic resection, liver transplantation, ablation therapy, and immunotherapy (Anwanwan et al., 2020). However, due to its insidious onset and lack of effective biomarkers, HCC patients are usually diagnosed in the mid- to late-stages (Yoh et al., 2021). It is therefore urgently necessary to identify validated diagnostic markers and establish strategies for the prognostic evaluation of HCC to improve the overall survival rate of patients.

Aldehyde dehydrogenase 2 (ALDH2) is an aldehyde oxidoreductase consisting of 517 amino acids that are encoded by the highly polymorphic ALDH2 gene, and a total of 280 variants of ALDH2 have been identified (Chen et al., 2020a). Of these,
seven variants, namely, ALDH2*1 (wild-type), ALDH2*2 (E504K mutant), ALDH2*3, ALDH2*4, ALDH2*5, ALDH2*6, and ALDH2*7, have been identified in more than 0.5% of patients, as determined from corresponding ethnic and geographic group frequencies (Chen et al., 2020a). ALDH2*2 is the most common genetic variation worldwide. The activity of the enzyme encoded by ALDH2*2 is significantly lower than that of the enzyme encoded by ALDH2*1 (Xu et al., 2014). Therefore, there are differences among the alcohol tolerance of populations carrying different alleles of ALDH2. Genetic polymorphisms in ALDH2 are prevalent in Asian populations, especially in China. It has been estimated that 40% of the Chinese population has a heterozygous genotype and 5% has the homozygous variant genotype (Ginsberg et al., 2002). Current reports estimate that approximately half a billion East Asians, constituting approximately 8% of the global population, harbor the ALDH2*2 allele (Matsumura et al., 2019; Zhang et al., 2023). Therefore, ALDH2 deficiency could be one of the most prevalent enzyme deficiencies in the human population.

An increasing number of studies have suggested that ALDH2 can modulate the onset and progression of HCC via multiple pathways. Polymorphisms in the ALDH2 gene are associated with an increased susceptibility to HCC. However, the ALDH2 enzyme has shown promising potential in the diagnosis, prognosis, and therapeutic management of HCC. However, the findings of current research studies are frequently inconsistent or contradictory owing to limited sample sizes and restricted research methods. Therefore, this review aimed to conduct a comprehensive survey of relevant literature in PubMed and the Web of Science database to identify relevant articles. Literature review was performed with a keyword-based search strategy by using various keywords, including “ALDH2” “gene polymorphism” “HCC” “hepatocellular carcinoma” and “susceptibility” either individually or in combination to ensure the inclusiveness of the literature review. The review outlines the correlation between ALDH2 and the development of HCC and discusses the potential application of ALDH2 as promising targets and biomarkers in clinical strategy.
2. Overview of HCC

HCC is a commonly occurring malignancy of hepatic epithelial cells and a major global health burden (Younossi et al., 2023). The development of HCC is historically associated with genetic predisposition as well as exposure to various environmental risk factors (Toh et al., 2023). There are substantial temporal and geographic variations in the onset and etiology of HCC. The risk factors for HCC have gradually shifted from viral factors to non-viral etiologies over time (Toh et al., 2023). This review focuses on the primary etiology underlying the development of HCC.

It has been reported that human hepatitis viruses are the predominant cause of HCC worldwide (Toh et al., 2023). A previous study demonstrated that more than 30% of individuals infected with hepatitis viruses exhibit chronic liver disease, which can advance to hepatitis, liver fibrosis, and cirrhosis, and can ultimately lead to the development of HCC (Arzumanyan et al., 2013). Several studies have demonstrated that individuals infected with HBV, and especially patients co-infected with HCV, are at an increased risk of developing HCC. High levels of HBV antigen and HBV DNA indicate an increase in HBV replication, which consequently leads to an increased risk of developing HCC (Kao et al., 2002). Timely vaccination is the most effective strategy for preventing infections caused by hepatitis viruses. Additionally, it is also regarded as the simplest and most effective strategy for mitigating the incidence of HCC and reducing the adult mortality rate.

The rate of progression and severity of liver disease caused by chronic alcohol consumption can vary substantially among individuals depending on the relative levels of alcohol intake, and this variation is attributed to the distinct expression profiles of ethanol-metabolizing enzymes across individuals (Gao and Bataller, 2011). Steatosis, steatohepatitis, and liver fibrosis are observed in the majority of individuals who indulge in the excessive consumption of alcohol for prolonged durations, of which approximately 10% of cases progress to cirrhosis, and 1–2% of cirrhotic patients eventually develop HCC (Seitz and Stickel, 2007). Therefore, the consumption of alcohol should be rigorously restricted, and further insights into the
correlation between ethanol intake and the occurrence of HCC are extremely necessary for mitigating the incidence of HCC.

The clinical feature of nonalcoholic fatty liver disease (NAFLD) is defined by the accumulation of fat within hepatocytes, which is a key contributor to the global prevalence of NAFLD as a chronic liver disease (Bedogni et al., 2023). NAFLD is a susceptibility factor for the development of HCC, and this is partly attributed to the role of the senescence-associated secretory phenotype (SASP) in the occurrence of obesity-associated HCC (Yoshimoto et al., 2013). Dietary or genetic obesity can alter the composition of gut microbiota, which in turn catalyze the metabolism of bile acids into deoxycholic acid (DCA). DCA triggers the generation of SASP by hepatic stellate cells (HSCs), which induces them to release of various inflammatory and tumor-promoting chemicals. This leads to the impairment of cellular DNA which can ultimately trigger the onset of HCC (Yoshimoto et al., 2013). A previous study investigated the role of the immune response in NAFLD-induced HCC by using rat models and human tissue samples (Ma et al., 2016). The findings revealed that the failure of lipid metabolism in NAFLD leads to a selective reduction in the population of intrahepatic CD4+ lymphocytes, which in turn accelerates hepatocellular carcinogenesis (Ma et al., 2016). Altogether, these studies provide insights into a possible novel therapeutic approach for the treatment of NAFLD-induced HCC in future.

3. Overview of ALDH2

ALDH2 is a mitochondrial enzyme that catalyzes the detoxification of acetaldehyde and endogenous aldehydes. It is a member of the aldehyde dehydrogenase family and comprises four identical subunits. The ALDH2 gene is located on chromosome 12 and consists of 13 exons and 12 introns (Wang et al., 2021). The gene encodes a protein of 517 residues, including a signal peptide of 17 residues (Zhang et al., 2023). The structural characteristics of the signal peptides of ALDH enzymes and other mitochondrial signal peptides are similar, with alternating arrangement of bases and hydrophobic residues. The primary function of ALDH2
involves the oxidation of acetaldehyde to non-toxic acetic acid to reduce the accumulation of acetaldehyde, which consequently protects the genetic material and reduces oxidative stress (Kimura et al., 2019).

It is reported that approximately 300 single nucleotide polymorphisms (SNPs) are associated with ALDH2 (Matsumoto et al., 2016b), of which 180 are substitution mutations (Matsumoto et al., 2016b). However, only the rs671 (ALDH2*2, E504K) SNP is associated with a major phenotypic change resulting from an alteration in the function of ALDH2. The mutation is located in exon 12 and results in the substitution of a glutamic acid residue at position 504 to a lysine residue. The substitution weakens the NAD binding site by preventing the formation of hydrogen bonds between E504 and R281 of the same subunit and R492 of the adjacent dimer partner in ALDH2 (Larson et al., 2005; Gong et al., 2013; Chang et al., 2017; Zhang et al., 2023). The glutamate residue at position 504 plays a key role in the formation of homodimers. Therefore, the substitution of this glutamate with lysine substantially decreases the activity of ALDH2 (Zhao and Wang, 2015). The activity of the ALDH2 enzyme encoded by the ALDH2*1*2 genotype is only 30–40% of that of the ALDH2 enzyme encoded by the ALDH2*1*1 genotype, while the enzyme encoded by the ALDH2*2*2 genotype has almost no activity (Chen et al., 2008). The blood concentrations of acetaldehyde in individuals with ALDH2*1*2 and ALDH2*2*2 genotypes are reported to be 5- and 18-fold higher, respectively, than those of individuals with the normal genotype, following the consumption of equal quantities of alcohol (Enomoto et al., 1991). The role of ALDH2 genes in specific environments and disease conditions is discussed hereafter in further detail.

4. Function role of ALDH2 in HCC

The emergence and progression of HCC is governed by several factors, including the immune system, tumor cell cycle, and genome stability, as well as the metastasis, proliferation, and resistance of cancer cells to multiple drugs, in which ALDH2 plays an important regulatory function. The role of ALDH2 in HCC and the therapeutic potential of targeting ALDH2 in HCC are discussed in detail in this section.
T cells are one of the primary subtypes of lymphocytes and constitute approximately 65–75% of the total lymphocyte population in peripheral blood (Melenhorst et al., 2022). T cells have various biological functions, including the direct elimination of target cells, induction or suppression of antibody production by B cells, response to specific antigens, and cytokine release (Liu and Sun, 2021). The activity of T cells is linked to the development of several human malignancies, including HCC (Woller et al., 2021; Chow et al., 2022). For instance, a previous study reported that CD8\(^+\) T lymphocytes play a protective role in HCC (Xu et al., 2019). In another study involving 446 HCC cases revealed that the density of CD8\(^+\) T lymphocytes in the core regions of malignant tissues and surrounding marginal areas was positively correlated with the overall survival (Sun et al., 2015). An increase in the density of CD8\(^+\) T lymphocytes was indicative of lower rates of disease recurrence. These results indicated that an increase in the density of CD8\(^+\) T lymphocytes may have a favorable prognostic impact in HCC. Similar to CD8\(^+\) T lymphocytes, CD4\(^+\) T lymphocytes also play a protective role in HCC (Scharping et al., 2021). A previous study demonstrated that the induction of apoptosis in CD4\(^+\) T lymphocytes promoted the development of HCC in a murine model of nonalcoholic steatohepatitis, whereas suppression of the apoptosis of CD4\(^+\) T lymphocytes prevented the development of HCC (Brown et al., 2018). These findings indicated that T lymphocytes could play a pivotal role in the pathogenesis, prognosis, and immunotherapeutic management of HCC. The expression levels of ALDH2 in patients with HCC are reported to be negatively related to the expression levels of programmed cell death 1 (PD-1) and cytotoxic T lymphocyte antigen-4 (CTLA-4) (Yao et al., 2021). Noteworthy, T cell receptors mediate antigen recognition by PD-1, and the interaction of PD-1 with PD-L1 expressed on the surface of tumor cells triggers a negative feedback regulatory pathway that attenuates the activity of T cells (Wu et al., 2022). CTLA-4 shares a high degree of homology with CD28 and competes with the latter for binding to B7 molecules present on the surface of antigen-presenting cells. The binding of CTLA-4 to B7 molecules could inhibit the activation of CD28, thereby suppressing the ability
of T cells to eliminate tumor cells (Zhang et al., 2021). ALDH2 may therefore significantly alter the activity of T lymphocytes by modulating PD-1 and CTLA-4, which in turn may modulate the progression of HCC.

Increasing evidence suggested a close relationship between the development of cancer and the genomic stability of tumor cells (Jamasi et al., 2022). The fundamental biological features of malignant cells include uncontrolled proliferation and apoptosis of tumor cells, which are attributed to the dysregulation of the cell cycle (Williams and Stoeber, 2012; Jeggo et al., 2016). Furthermore, the development of HCC is a multifaceted and multistep process associated with a complex genetic basis. The impairment of genomic stability can trigger the activation of numerous oncogenic pathways that ultimately leads to the onset and progression of HCC (Kessler et al., 2015). Additionally, the dysregulation of cell cycle-related genes may promote the progression of HCC by disrupting cellular proliferation and apoptosis (Cheng et al., 2007). It has been reported that the cell cycle is regulated by ALDH2 (Ebert et al., 2014), and a previous study demonstrated that mice harboring the ALDH2*2 allele exhibited an aberrant increase in the expression level of E2F transcription factor 1 (E2F1), which can promote the advancement of HCC (Yao et al., 2021). E2F1 is associated with cell cycle regulation and predominantly participates in controlling a diverse range of cellular functions, including cell cycle progression, cellular proliferation, and apoptosis (Shen et al., 2020). Clinical data indicated that the expression level of E2F1 is elevated not only in HCC, but are also correlated with unfavorable clinicopathological characteristics and a relatively poor prognosis (Yao et al., 2021). Subsequent in vivo experiments in mice and in vitro cellular experiments demonstrated that E2F1 induces the progression of HCC by enhancing the expression of stathmin 1 (STMN1) (Chen et al., 2013). The oncogenic significance of STMN1 in various types of cancers has been extensively documented. STMN1 alters cell cycle progression in HCC by modulating cellular microtubule dynamics and promoting the depolymerization of intermitotic microtubules by binding to microtubulin dimers which in turn destabilizes the microtubules (Wang and Yang, 2021). Therefore, these
findings indicated that ALDH2 can potentially influence the development of HCC by modulating the cell cycle. Several studies have reported that ALDH2 can also modulate genomic stability (Kunugita et al., 2008; Garaycoechea et al., 2018; Seo et al., 2019). For instance, a previous study observed that the frequency of sister chromatid exchange increased by 2.3-fold in mice harboring the ALDH2*2*2 genotype compared to that of the control group (Mu et al., 2021). Another study demonstrated that chronic alcohol consumption increased the expression levels of oxidative stress in mice harboring the ALDH2*2 allele owing to the inadequate activity of ALDH2, which increased the production of reactive oxygen species (ROS) (Lee et al., 2008). Meanwhile, it has been reported that the expression of cytochrome P450 (CYP2E1) is upregulated in ALDH2-deficient mice (Matsumoto et al., 2016a). CYP2E1 also increases ROS production during the metabolism of certain low-molecular-weight compounds, including ethanol and fatty acids (Matsumoto et al., 2016a). ROS can induce structural changes in DNA by targeting both single- and double-stranded regions, which leads to base pair mutations or alterations in the deoxyribose moiety of DNA in HCC. Furthermore, ROS can also interact with biological macromolecules such as DNA to induce the generation of DNA adducts, which reduces the genomic stability (Jin et al., 2015). DNA adducts can be potentially transported to adjacent hepatocytes via extracellular vesicles and may activate several oncogenic pathways, including the signal transducer and activator of transcription 3 (STAT3), C-Jun N-terminal kinase (JNK), transcriptional co-activator with PDZ-binding motif (TAZ), and BCL-2 protein (Seo et al., 2019), which can ultimately promote the development of HCC. ALDH2 can therefore potentially alter the cell cycle in HCC by regulating the expression of E2F1 and altering genomic stability by modulating ROS production. These mechanisms may in turn alter the onset and development of HCC.

Cancer stem cells (CSCs) are a subset of liver cancer cells having stem cell-like properties that determine the graded organization of liver cancer cells and are linked to treatment resistance and tumor recurrence (Huang et al., 2020). CSCs have been
identified as crucial contributors to tumor initiation, invasion, metastasis, recurrence, and chemoresistance in HCC (Liu et al., 2015; Liu et al., 2020). Previous studies have demonstrated that certain prominent cell surface markers, including CD44 and CD133, can be used to identify hepatic CSCs (Li, 2013; Asai et al., 2019). It has been further reported that these markers are implicated in the pathogenesis of CSCs in HCC via the AKT/GSK-3β, AKT-pKB, and TGF-β signaling pathway (Jeng et al., 2023). CD44 and CD133 belong to the family of transmembrane glycoproteins (Zheng et al., 2014), and a previous study suggested that high expression levels of CD44 are associated with the worsening of survival prognosis in patients with HCC (Guo et al., 2014). Additionally, several studies have reported that there is a significant association between the expression level of CD133 and the clinicopathological features of HCC (Suetsugu et al., 2006; Yamashita et al., 2009). A previous study reported that individuals with high expression levels of CD133 had a lower 5-year overall survival rate (Song et al., 2008). Altogether, these findings indicated that the surface markers expressed by CSCs can modulate the development of HCC by activating distinct signaling pathways. Therefore, these results suggested that ALDH enzymes can serve as novel therapeutic targets for combating chemoresistance in CSCs. It has been reported that ALDH inhibitors can enhance the responsiveness of cancer cells to chemotherapy agents (Moreb et al., 2017). Additionally, ALDH2 can upregulate the expression level of biomarkers of CSCs, including CD44 and CD133, suggesting that ALDH2 may promote metastasis, proliferation, apoptosis, and chemoresistance in HCC by regulating hepatic cancer stem cells (HCSCs). Consistent with this hypothesis, a previous study demonstrated that knockdown of ALDH2 suppresses metastasis and proliferation in HCC and promotes tumor cell apoptosis (Chen et al., 2020b). Therefore, ALDH2 may induce metastasis, proliferation, apoptosis, and chemoresistance in HCC by regulating HCSCs, which ultimately affects the development of HCC.

In conclusion, these findings suggested that ALDH2 is aberrantly expressed during the progression of HCC. The majority of studies indicated that suppression of
the expression of ALDH2 contributes to the advancement of HCC. Contrarily, some studies have suggested that ALDH2 silencing may inhibit the progression of HCC. Specifically, ALDH2 inhibits the progression of HCC via T lymphocytes and hepatoma cells. However, ALDH2 can promote the progression of HCC by regulating HCSCs (Figure 1). These findings imply that manipulation of the expression level of ALDH2 could serve as a promising strategy in preventing the progression of HCC. It is therefore essential to investigate the precise regulatory mechanisms for developing therapeutic regimens for HCC.

5. Regulatory mechanism of ALDH2 in HCC

The treatment of cancer is difficult due to the complex processes of tumorigenesis and metastasis. Effective biomarkers such as ALDH2 have been identified for determining the occurrence and metastasis of cancer mediated by direct or indirect modes of regulation. However, the precise molecular mechanisms by which ALDH2 regulates the progression of HCC remain to be elucidated. HCC is intricately linked to the aberrant activation of the adenosine monophosphate-activated protein kinase (AMPK) and the mammalian target of rapamycin (mTOR) signaling pathways (Feng et al., 2020; Lou et al., 2020). This section provides an extensive overview of the regulatory mechanisms by which ALDH2 regulates the progression of HCC in view of the intricate interactions of the AMPK and mTOR signaling pathways (Figure 2).

5.1 AMPK signaling pathway

AMPK is a highly conserved heterotrimeric protein complex consisting of α, β, and γ subunits, and is expressed in various eukaryotic organisms (Carling, 2017). AMPK was first described in relation to its role in lipid metabolism and regulation of cholesterol and fatty acid levels (Li et al., 2015). In addition to its role in energy regulation, AMPK modulates various vital cellular physiological processes (Luo et al., 2005).

Increasing evidence demonstrated that the AMPK signaling pathway plays a pivotal regulatory role in the progression of HCC (Shui et al., 2020). The inhibitory
effects of adenine on the growth of HCC cells were investigated in a previous study, and the findings revealed that treatment with adenine substantially reduced the expression levels of cyclin A, cyclin D1, and cyclin-dependent kinases 2, and increased the expression levels of Bcl2-associated X, NOXA, and p53-upregulated modulator of apoptosis in HCC cells (Su et al., 2020). Treatment with adenine also induced the cleavage of Caspases-3 and 9. Investigation of the potential mechanism underlying the inhibitory effects of adenine on HCC further revealed that adenine inhibits the growth of HCC cells by activating the AMPK-mediated p53 signaling pathway (Su et al., 2020). Another study investigated the effects of treatment with isoquercitrin (ISO) on the death of liver cancer cells, and the results demonstrated that treatment with ISO induced apoptosis and autophagy in HepG2 and Huh7 cells (Shui et al., 2020). The possible role of autophagy and its relation to the ISO-induced apoptosis of HCC cells were additionally investigated. In vitro cellular experiments revealed that treatment with ISO activated AMPK and suppressed the mTOR/p70S6K signaling cascade in HCC cells. This induced the upregulation of key autophagy-related proteins, including Atg5, Beclin-1, and LC3B-II, and promoted the formation of autophagosomes, which led to cytotoxic effects in HCC cells. These studies suggested that the activation of the AMPK signaling pathway can exert an inhibitory effect on HCC cells by promoting fatty acid oxidation and autophagy.

Emerging research in the field of genomics demonstrated that the proliferation, invasion, and migration of HCC cells can be effectively inhibited by targeting the ALDH2 protein (Chen et al., 2022). In order to investigate the molecular mechanisms underlying the inhibition of ALDH2 in HCC, a study analyzed the expression profiles of HCCLM3-ALDH2 cells by using a gene microarray platform (Hou et al., 2017). The result of ingenuity pathway analysis revealed that the expression levels of the proteins in the AMPK signaling pathway, including CREB5, CREB3, and CHRNE, were significantly altered. The findings further revealed that overexpression of ALDH2 activated AMPK phosphorylation, while knockdown of ALDH2 inhibited AMPK phosphorylation. These results suggested that ALDH2 hinders the progression
of HCC via AMPK signaling pathway. Notably, HCC cells were treated with activators (metformin) and inhibitors (compound C) of AMPK to investigate whether the activation of p-AMPK is essential for the ALDH2-induced HCC cells. The findings revealed that metformin significantly increased the inhibitory effect of ALDH2 in HCC cells, whereas knockdown of AMPK significantly decreased the inhibitory effect of ALDH2 in HCC cells (Hou et al., 2017). These studies indicated that ALDH2 can potentially suppress the metastasis of HCC cells by activating AMPK signaling pathway. Additionally, the AMPKα subunit possesses redox-sensitive cysteines that are readily oxidized in response to oxidative stress (Shao et al., 2014). It is therefore plausible to speculate that ALDH2 induces the phosphorylation of AMPK via a redox-dependent mechanism, which contributes to the inhibitory effect of ALDH2 on the progression of HCC.

Based on these findings, it can propose that ALDH2 might induce the oxidation of cysteine residues in the AMPKα subunit by regulating the in vivo redox status. This would lead to the activation of the AMPK signaling pathway, and in turn might inhibit the progression of HCC by modulating the proliferation, migration and autophagy of HCC tumor cells.

5.2 mTOR signaling pathway

The mTOR tyrosine kinase is localized in the cytoplasm and is involved in the regulation of various physiological processes, including cell growth, metabolism, and survival (Saxton and Sabatini, 2017). Strong evidence suggests that the overactivation of the mTOR signaling pathway is associated with the development of a range of cancers, including HCC (Murugan, 2019; Wu et al., 2020b).

In order to elucidate the potential regulatory role of the mTOR signaling pathway in the progression of HCC, Zahid and coworkers analyzed the gene expression data of patients with HCC (Zahid et al., 2019). The genes that were downregulated during the onset and progression of HCC were markedly overrepresented in patients with low expression levels of the mTOR protein. Additionally, the expression levels of the mTOR protein were found to be higher in HCC tumor cells. The reported survival
outcomes of patients with HCC in the TCGA database were found to be associated with the expression levels of mTOR and various upstream regulators and downstream effectors in the mTOR signaling pathway (Zahid et al., 2019). Tian et al. reviewed the role of the mTOR signaling pathway in the metabolic processes of HCC cells (Tian et al., 2023), and discussed the underlying mechanisms by which the mTOR signaling pathway regulates various metabolic processes in HCC, including glucose metabolism, lipid metabolism, oxidative metabolism, lactic acid metabolism, and amino acid metabolism. Specifically, the activation of the mTOR signaling pathway induces the expression of several metabolic enzymes involved in glycolysis, lipogenesis, and amino acid synthesis, which in turn enhances the anabolic development of tumors during metabolic reprogramming. These findings suggested that the mTOR signaling pathway is a key factor in the pathogenesis of HCC. However, further studies are necessary for elucidating the potential mechanisms underlying the regulatory role of the mTOR signaling pathway in the progression of HCC.

Zahid et al. performed enrichment analysis using gene sets representing different metabolic pathways (Zahid et al., 2019). The findings revealed that the gene sets that were associated with low expression levels of mTOR were significantly enriched, implying that the metabolic processes downstream of the mTOR signaling pathway were suppressed. In order to identify the functional targets of the mTOR signaling pathway, the metabolic genes that were downregulated in HCC were initially screened in the study. Analysis of data retrieved from the TCGA database and multiple Gene Expression Omnibus datasets revealed a negative correlation between the mTOR signaling scores and expression levels of ALDH2. Immunohistochemistry analysis of HCC tissues further revealed that the expression levels of mTOR and ALDH2 proteins were inversely associated (Zahid et al., 2019). Altogether, these findings imply that the regulation of ALDH2 might occur downstream of the mTOR signaling pathway. Further analyses were performed to elucidate the mechanism by which the mTOR signaling pathway regulates the expression of ALDH2. The findings revealed that histone deacetylase 1 (HDAC1) plays a critical role in modulating the expression
levels of ALDH2. Moreover, the ribosomal protein S6 kinase 1 (S6K1) protein phosphorylates the downstream effectors of the mTOR signaling pathway to increase the activity of HDAC1 (Citro et al., 2015). The study by Zahid et al. reported that activation of the mTOR signaling pathway and expression levels of S6K1 protein and HDAC1 protein were positively associated with the development of HCC (Zahid et al., 2019). However, the HDAC1 activity score was found to be negatively associated with the expression level of ALDH2 and the survival rate of patients with HCC. The findings additionally revealed that treatment with an HDAC1 inhibitor upregulated the expression level of ALDH2 in HCC cells (Zahid et al., 2019).

Based on these studies, the overactivation of the mTOR signaling pathway could increase the activity of HDAC1 via S6K1, which can suppress the expression of ALDH2 and ultimately modulate the development of HCC.

6. Relationship between ALDH2 gene polymorphisms and HCC susceptibility

There may be a correlation between ALDH2 gene polymorphisms and cancer susceptibility, such as colorectal cancer, esophageal cancer, gastric cancer, and HCC (Duell et al., 2012; Yukawa et al., 2012; Singh et al., 2015). The relationship between ALDH2 gene polymorphisms and susceptibility to other cancers has been identified by several studies (Yang et al., 2009; Hidaka et al., 2015). Additionally, existing studies have showed that the association of ALDH2 gene polymorphisms with HCC susceptibility was still controversial (Table 1).

Some studies have suggested that ALDH2 gene polymorphisms was associated with HCC susceptibility (including increased or decreased susceptibility to HCC). Moreover, studies have suggested that the effect of ALDH2 gene polymorphisms on HCC susceptibility might be influenced by risk factors, particularly alcohol consumption (Sakamoto et al., 2006; Ding et al., 2008; Liu et al., 2016). For instance, a study conducted in Jiangsu Province, China, revealed that drinkers with the ALDH2*1*2 or ALDH2*2*2 genotypes had a significantly higher susceptibility to HCC compared to non-drinkers with the ALDH2*1*1 genotype (OR = 3.30; 95% CI = 1.24–8.83) (Ding et al., 2008). Similarly, another study conducted in Japan
demonstrated that ALDH2 gene polymorphisms increased susceptibility to HCC among light to moderate drinkers (OR = 4.5 or 2.0; 95% CI = 1.2–16.5)(Sakamoto et al., 2006). Additionally, the association of ALDH2 gene polymorphisms with HCC susceptibility may also be influenced by HBV or HCV (Li et al., 2016). For example, some studies have demonstrated that susceptibility to HCC was elevated in individuals with the ALDH2*2 allele and simultaneously infected with HBV or HCV (OR = 1.64; 95% CI = 1.03–2.60)(Tomoda et al., 2012; Chien et al., 2016). Notably, a study conducted in Guangxi Province, China, suggested that rs671 (ALDH2*2, E504K) could act as protective factor against susceptibility to HCC (OR = 0.20; 95% CI = 0.06–0.68)(Ye et al., 2018). Therefore, clinically, some reasonable treatment recommendations could be given to patients according to their ALDH2 genotypes, in order to achieve the goal of individual precision medicine.

However, some studies have suggested that there was no association between ALDH2 gene polymorphisms and HCC susceptibility. For instance, a Meta-analysis conducted by Zhou et al. involving 1,231 patients of HCC and 1,849 controls revealed that ALDH2 gene polymorphisms was not associated with HCC susceptibility (OR = 0.99; 95% CI = 0.72–1.34)(Zhou et al., 2012). Similarly, a study in Japan involving 102 patients of HCC and 125 controls reported that ALDH2 gene polymorphisms was not associated with susceptibility to HCC (OR = 1.10; 95% CI = 0.60–2.10)(Takeshita et al., 2000).

In summary, there was still controversy on the relationship between ALDH2 gene polymorphisms and HCC susceptibility, which may be attributed to inadequate sample sizes and different research methods. Additionally, ALDH2 gene polymorphisms exhibit geographic variations (Table 2), which could also affect the results of investigating the relationship between ALDH2 gene polymorphisms and HCC susceptibility. Therefore, in order to elucidate this relationship between ALDH2 gene polymorphisms and HCC susceptibility, large amounts of statistical data-analysis and the application of more effective scientific methods were still needed to proceed.

7. Potential clinical applications of ALDH2 in HCC
Owing to the inconspicuous clinical symptoms and insidious onset, patients with HCC are frequently diagnosed in the intermediate to advanced stages of the disease, which contributes to the lower overall survival rate. It has been additionally reported that patients have suboptimal prognosis and high recurrence rates despite receiving treatment (Fujiwara et al., 2018). It is therefore essential to identify effective diagnostic and prognostic markers for early diagnosis and prediction of patient survival, which may aid in selecting a suitable therapy program. This review discusses the feasibility of applying the expression levels of ALDH2 in the clinics for the diagnosis, prognostic assessment, and treatment of HCC. This section describes the potential clinical applications of ALDH2 in diagnosing and predicting the prognosis of HCC.

Previous studies have demonstrated that the expression levels of ALDH2 mRNA and protein are significantly decreased in HCC tumors, and the expression levels are further pronounced in highly aggressive tumors (Park et al., 2002; Yao et al., 2022). By quantitative real-time polymerase chain reaction (PCR) analysis of 92 HCC tumor tissues, the study demonstrated that the expression level of ALDH2 was notably lower in HCC tumors than in adjacent non-tumor tissues in the majority of samples (77.2%) (Hou et al., 2017). Subsequent RNA-seq analysis of these HCC samples revealed that the expression levels of ALDH2 in 98% of the tumor tissues were significantly lower than those in the adjacent non-tumor tissues. Additionally, the results of immunoblot analysis and immunohistochemistry staining consistently demonstrated that the expression levels of ALDH2 were lower in the HCC tumor tissues. These findings imply that the expression level of ALDH2 may serve as a valuable biomarker for the diagnosis of HCC. Numerous studies have additionally reported that there is a relation among the expression level of ALDH2, tumor grade, and clinical stages of patients. Specifically, the expression level of ALDH2 was decreased with the advancement of the tumor stage and clinical stage. A study on 374 patients with HCC (Yao et al., 2022) revealed that 48.4% of patients with low levels of ALDH2 expression had mid-to-late stage tumors, while only 25.6% of patients with
high levels of ALDH2 expression had early stage tumors. The study further assessed the prognostic significance of the expression levels of ALDH2 by using a receiver operating characteristic curve, which revealed that the area under the curve was 0.925. Altogether, these findings revealed that ALDH2 can serve as a promising prognostic biomarker for HCC. In addition to serving as a marker for assessing patient prognosis, polymorphisms in the ALDH2 gene have also been found to be intricately associated with patient outcomes. It has been reported that individuals harboring the ALDH2*2 allele are more likely to abstain from alcohol following diagnosis due to alcohol intolerance, and these patients tend to have a better prognosis. However, patients harboring the ALDH2*1*1 genotype are less likely to abstain from alcohol owing to higher alcohol tolerance, and these individuals tend to have a poorer prognosis (Huang et al., 2019). This suggested that better treatment outcomes can be achieved for diagnosed individuals by modulating the consumption of alcohol.

These findings demonstrated that ALDH2 may serve as a suitable marker for the early diagnosis, prognostic assessment, and modulating the alcohol consumption of patients with HCC. Despite the remarkable progress of current studies, it is necessary to further explore the specific role of ALDH2 in the development of HCC and determine its interrelationship with other biomarkers for improving the precision and accuracy of early diagnosis and prognostic assessment of HCC. Additional prospective studies are necessary for validating the practical application of ALDH2 in the diagnosis and prognostic assessment of HCC.

8. Future perspectives

ALDH2 is an important cellular detoxification enzyme and its relationship with cancer has been studied extensively. However, the precise association between ALDH2 and HCC is not completely understood. Targeted therapies and gene editing approaches may offer promise in the treatment of HCC. Previous treatment strategies for HCC involved the targeting of tumor-associated macrophages, and gene editing approaches have been used to repair any DNA damages to genes encoding proteins in the ALDH family (Lian et al., 2018). Further in-depth studies are therefore necessary.
for exploring potential ALDH2-based treatment approaches for HCC.

Targeted therapies for HCC have progressed immensely and have become a focus of clinical research. The current targeted therapies for HCC primarily include molecular targeted therapies and organ targeted therapies (Zhang et al., 2022). A previous study demonstrated that alcohol oxidase combined with hemin-conjugated chitosan and oral ethanol can inhibit the proliferation of hepatoma cells by triggering intracellular ferroptosis (Hao et al., 2022). Furthermore, when immobilized in the liver using alginate hydrogels formed in situ, the therapeutic efficacy of this combination was found to be comparable to traditional percutaneous ethanol injection, a targeted therapy administered at the organ level. Additionally, it has been demonstrated that non-viral vectors, lipid nanoparticles, and virus-like particles can serve as delivery vehicles to overcome various physiological barriers in the body (Tang et al., 2021). These findings suggested that cellular-level targeted therapies could be explored for the treatment of HCC. It has been reported that high expression levels of ALDH2 in HCC tumor tissues are beneficial for the treatment and prognosis of HCC. However, high expression levels of ALDH2 in HCCSCs can promote the metastasis and proliferation of HCC cells and thereby induce the progression of HCC. Therefore, the development of a cellular-level targeted therapy that maintains high expression levels of ALDH2 in HCC cells and low expression level of ALDH2 in HCCSCs offers a promising avenue of research for the treatment of HCC.

Gene editing refers to the targeted modification of target genes, and involves various methods, including knockout, replacement, and correction, to obtain new functions or phenotypes. CRISPR/Cas9 is a third-generation gene editing technology that is widely employed in HCC due to its exceptional gene editing efficiency and precision (Wu et al., 2020a). A previous study reported that HCC can be effectively treated by manipulating the cancer genome using CRISPR/Cas9 technology (Qi et al., 2020). By employing the CRISPR/Cas9 technology for multiplex genome engineering, Xue et al. observed that the metabolism of ethanol can be regulated by modifying ALDH2 and ALDH4 (Xue et al., 2018). Notably, the CRISPR/Cas9 technology was
used in a previous study to construct ALDH2-knockout HepG2 cell lines (Wang et al., 2018). These studies indicate that the CRISPR/Cas9 technology can be used to modify the human ALDH2 gene and may serve as a potential novel therapeutic strategy for HCC.

In summary, cellular-level targeted therapies and gene editing technologies show promise in the treatment of HCC, and the findings of existing reports indicate that ALDH2 is a suitable therapeutic target for HCC.

9. Conclusions

ALDH2 plays a regulatory role in various cancers, including HCC. Numerous studies have demonstrated that ALDH2 plays an important role in the metastasis, proliferation, migration, invasion, diagnosis, and prognosis of HCC. ALDH2 participates in the regulation of signaling pathways related to HCC, and polymorphisms in the ALDH2 gene are closely associated with susceptibility to HCC. The findings indicated that ALDH2 plays a key regulatory role in the development of HCC. Studies involving larger samples and more precise techniques are necessary for determining the possible clinical applications of ALDH2 for the treatment of HCC in future.

Data Availability Statement

The authors declare that no data was presented in this manuscript.

Figure Legends

Figure 1. Functional role of ALDH2 in HCC.

(a) ALDH2 modulates the activity of T cells in liver cancer via PD-1 and CTLA-4 to promote the immune escape of HCC cells. (b) ALDH2 induces metastasis, proliferation, and multidrug resistance in HCC by regulating HCSCs. (c) ALDH2 activates STMN1 by increasing the expression of E2F1. This in turn alters the cell cycle by inducing tubulin depolymerization, modulating the proliferation and apoptosis of HCC cells. (d) ALDH2 alters genome stability by inducing ROS
production, which promotes the occurrence and development of HCC.

**Figure 2. Regulatory mechanism of ALDH2 in HCC**

ALDH2 activates the AMPK signaling pathway by altering the cellular redox status, and suppresses the progression of HCC by modulating cellular proliferation, growth, and autophagy. Additionally, the overactivation of mTORC1 leads to the phosphorylation of HDAC1 via the regulation of S6K1, which in turn increases the activity of HDAC1. The development of HCC is further promoted via the inhibition of ALDH2 expression.

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**Conflict of interest**
We affirm that there are no known conflicts of interest in this paper, and there is no substantial financial support for this research that could potentially influence its outcomes.

**Author Contributions**

Wrote or contributed to the writing of the manuscript: Dashuai Yang, Ying Hu, Junfa Yang, Liangsong Tao, Yue Su, Yincui Wu, Yan Yao, Shuxian Wang, Sheng Ye, Tao Xu.
Table 1  The effect of ALDH2 genotype on HCC susceptibility

<table>
<thead>
<tr>
<th>ALDH2 genotype</th>
<th>Effect on HCC</th>
<th>Sample</th>
<th>OR</th>
<th>95% CI</th>
<th>Ref. (PMID)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALDH2<em>1</em>1</td>
<td>-  ^a</td>
<td>HCC patients (248)</td>
<td>1.38</td>
<td>0.86-2.23</td>
<td>12495467</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and controls (248)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALDH2<em>1</em>1</td>
<td>-  ^b</td>
<td>HCC patients (84)</td>
<td>1.24</td>
<td>0.70-2.20</td>
<td>12718671</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and controls (84)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALDH2<em>1</em>2</td>
<td>-  ^c</td>
<td>HCC patients (85)</td>
<td>1.1</td>
<td>0.60-2.10</td>
<td>10737710</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and controls (101)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ALDH2<em>2</em>2</td>
<td>-  ^d</td>
<td>HCC patients (85)</td>
<td>0.8</td>
<td>0.50-1.50</td>
<td>10737710</td>
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</table>

**Notes:**
- ^a^ indicates no significant influence on HCC.
- ^b^ indicates promotes HCC.
- ^c^ indicates restrain HCC.
- ^d^ indicates 95% CI not mentioned in the reference.
Table 2  ALDH2 gene polymorphisms and HCC susceptibility in different regions

<table>
<thead>
<tr>
<th>ALDH2*1 (frequency)</th>
<th>ALDH2*2 (frequency)</th>
<th>Sample size (HCC)</th>
<th>Common Mutation Types</th>
<th>Incidence of HCC (/100000)</th>
<th>Region</th>
<th>Ref. (PMID)</th>
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</thead>
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<tr>
<td>81.25%</td>
<td>18.75%</td>
<td>592</td>
<td>rs671</td>
<td>18.2</td>
<td>China</td>
<td>29765251</td>
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<tr>
<td>81.69%</td>
<td>18.31%</td>
<td>3821</td>
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<td></td>
<td></td>
<td>26827895</td>
</tr>
<tr>
<td>83.19%</td>
<td>16.81%</td>
<td>415</td>
<td></td>
<td></td>
<td></td>
<td>18439068</td>
</tr>
<tr>
<td>79.14%</td>
<td>20.86%</td>
<td>266</td>
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<td>12495467</td>
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<tr>
<td>96%</td>
<td>4%</td>
<td>134</td>
<td>rs671</td>
<td>10.4</td>
<td>Japan</td>
<td>25778454</td>
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<tr>
<td>83.42%</td>
<td>16.58%</td>
<td>463</td>
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<td></td>
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<td>22004425</td>
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<tr>
<td>82.85%</td>
<td>17.15%</td>
<td>484</td>
<td></td>
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<tr>
<td>66.74%</td>
<td>33.26%</td>
<td>227</td>
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<td>12940444</td>
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<tr>
<td>73%</td>
<td>27%</td>
<td>218</td>
<td>rs671</td>
<td>14.3</td>
<td>Korea</td>
<td>1733836</td>
</tr>
<tr>
<td>84%</td>
<td>16%</td>
<td>481</td>
<td></td>
<td></td>
<td></td>
<td>9309300</td>
</tr>
<tr>
<td>100%</td>
<td>0%</td>
<td>105</td>
<td>rs4767939</td>
<td>6.9</td>
<td>USA</td>
<td>10091951</td>
</tr>
<tr>
<td>100%</td>
<td>0%</td>
<td>61</td>
<td>rs2238151</td>
<td></td>
<td></td>
<td>1733836</td>
</tr>
<tr>
<td>100%</td>
<td>0%</td>
<td>193</td>
<td>rs886205</td>
<td>6.2</td>
<td>Europe</td>
<td>1733836</td>
</tr>
<tr>
<td>98.7%</td>
<td>1.3%</td>
<td>117</td>
<td>rs968529</td>
<td></td>
<td></td>
<td>1733836</td>
</tr>
</tbody>
</table>
Figure 1

**a. Immune system**
- Antigen presenting cell
- B7
- CTLA-4
- CD-28
- T cell
- CD133
- CD44
- ALDH2
- TCR
- PD-1
- PD-L1
- Liver tumor cell

**b. Metastasis**
- Proliferation
- Multidrug resistance
- Apoptosis
- Metastasis
- AKT/pKB
- TGF-β
- CD133
- CD44
- ALDH2
- Cancer stem cell

**c. Cell cycle**
- ALDH2
- ALDH2*2
- E2F1
- STMN1
- Microtubule depolymerization
- G2
- M
- S
- 12 hrs
- G1

**d. Stability of genome**
- ALDH2
- CYP2E1
- ROS
- DNA
- DNA adduct
- STAT3
- JNK
- TAZ
- BCL-2

Hepatocyte

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Figure 2

HBV → PI3K → AKT → mTOR → S6K1 → ALDH2

HCV → ALDH2

HDV → ALDH2

Alcohol → Acetaldehyde → AMPKα

High fat diet → Acetate → CREB5, CREB3, CHRNE

Promote:
- Lactic acid metabolism
- Amino acid metabolism
- Glucose metabolism
- Oxidative metabolism
- Lipid metabolism

Inhibit:
- Cyclin A
- Cyclin D1
- CDK2
- Bax
- NOXA
- PUMA
- Atg5
- Beclin-1
- LC3B-II

Hepatocyte