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CANCER INNOVATION

ORIGINAL ARTICLE

Expression pattern and prognostic significance of aldehyde dehydrogenase 2 in lung adenocarcinoma as a potential predictor of immunotherapy efficacy

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Abstract

Background: The incidence of alcohol-associated cancers is higher within Asian populations having an increased prevalence of an inactivating mutation in aldehyde dehydrogenase 2 (*ALDH2*), a mitochondrial enzyme required for the clearance of acetaldehyde, a cytotoxic metabolite of ethanol. The role of alcohol consumption in promoting lung cancer is controversial, and little attention has been paid to the association between alcohol drinking and pulmonary ALDH2 expression.

Methods: We performed a comprehensive bioinformatic analysis of multi-omics data available in public databases to elucidate the role of ALDH2 in lung adenocarcinoma (LUAD).

Results: Transcriptional and proteomic data indicate a substantial pulmonary expression of ALDH2, which is functional for the metabolism of alcohol diffused from the bronchial circulation. ALDH2 expression is higher in healthy lung tissue than in LUAD and inhibits cell cycle, apoptosis, and epithelial–mesenchymal transition pathways. Moreover, low *ALDH2* mRNA levels predict poor prognosis and low overall survival in LUAD patients. Interestingly, ALDH2 expression correlates with immune infiltration in LUAD.

Conclusions: A better understanding of the role of ALDH2 in lung tumor progression and immune infiltration might support its potential use as a prognostic marker and therapeutic target for improving immunotherapeutic response.

Abbreviations: ADH, alcohol dehydrogenase; ALDH2, aldehyde dehydrogenase 2 family member; ALDH2*2, rs671 polymorphism (Glu504Lys) of aldehyde dehydrogenase 2; corGSEA, correlation-based Gene Set Enrichment Analysis; CYP2E1, cytochrome P450 2E1; GEPIA, gene expression profiling interactive analysis platform; GTEx, Genotype-Tissue Expression project; ICI, immune checkpoint inhibitor; LDHD, lactate dehydrogenase D; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; NSCLC, non-small cell lung cancer; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; TCGA, The Cancer Genome Atlas; TIGIT, T cell immunoreceptor with Ig and ITIM domains; TIME, tumor immune microenvironment; ToPP, Tumor online Prognostic analyses Platform; TPM, transcripts per million; UALCAN, University of Alabama at Birmingham Cancer Data Analysis Portal.

Silvia Baldari and Gabriele Toietta shared the corresponding authorship.

KEYWORDS
acetaldehyde, alcohol, aldehyde dehydrogenase, ethanol metabolism, immunotherapy, lung adenocarcinoma, mitochondria, rs671 polymorphism (Glu504Lys) of aldehyde dehydrogenase 2

1 | INTRODUCTION

Alcohol consumption increases the risk of several forms of cancer and accounts for more than 4% of new cancer cases worldwide [1]. Several mechanisms may sustain alcohol-associated carcinogenesis, including the induction of oxidative and endoplasmic reticulum stress [2], depletion of folate [3], induction of stem cell damage [4], interference with retinoids and estrogen levels [5], alteration of the cell proliferation rate [6], and epigenetic changes [7]. However, increasing evidence points to acetaldehyde, a metabolite of ethanol, as one of the major determinants of alcohol's carcinogenic effect, mainly through the production of acetaldehyde-derived DNA adducts [8, 9]. Sources of acetaldehyde may be natural, such as plants and fermented foods, as well as anthropogenic, including incomplete combustion of organic biomass and fuels, building products, furniture, cleaners, cosmetics, and glues [10]. The major lifestyle-related risk factors increasing lung exposure to acetaldehyde are smoking and drinking alcoholic beverages [11]. Ingested ethanol is metabolized in the body by alcohol dehydrogenase (ADH), catalase, or cytochrome P450 2E1 (CYP2E1) to acetaldehyde, which is then further oxidized by aldehyde dehydrogenase 2 (ALDH2) to acetate. This process generates reactive oxygen species (ROS) that may induce DNA damage [12]. Moreover, acetaldehyde leads to the formation of DNA adducts, thus inhibiting DNA repair systems and interfering with DNA replication. Therefore, to prevent harmful effects, it is critical to detoxify aldehydes that may accumulate through endogenous metabolism and environmental exposure.

The family of aldehyde dehydrogenase enzymes is critical in the detoxification of endogenous and exogenous aldehyde substrates, in the biosynthesis of vital biomolecules, such as retinoic acid and folate, and in redox balance. Consequently, altered ALDH activity has been associated with increased vulnerability to different pathologies, including cancer [13]. Nineteen isoforms of ALDHs have been characterized in humans, showing different tissue and subcellular distributions and substrate specificity [14]. The mitochondrial enzyme ALDH2 is the most efficient isoform for converting toxic acetaldehyde to harmless acetate. In addition, ALDH2 detoxifies other aldehydes, such as 4-hydroxynonenal, an endogenous product of lipid peroxidation, and methylglyoxal, a glycolysis metabolite, acting as a protector against oxidative stress [15] and advanced glycation end products formation [16].

Lung cancer is one of the most frequent tumors and the most common cause of cancer death, accounting for approximately one-quarter of all cancer mortality worldwide [17]. Non-small cell lung cancers (NSCLCs) have been traditionally classified as lung adenocarcinoma (LUAD), which is the prevalent form comprising 40% of lung cancers, and lung squamous cell carcinoma (LUSC). Nonetheless, increasing evidence suggests that LUAD and LUSC should be considered as distinct diseases at the molecular, pathological, and clinical levels [18]. Current therapeutic approaches mainly rely on surgical resection, chemotherapy, radiotherapy, and, more recently, immunotherapy. Progressive improvements in the molecular characterization of lung cancer have led to the development of novel diagnostic approaches and targeted therapies. Nonetheless, patients' 5-year survival rates remain below 20%. Immune checkpoint inhibitor (ICI) therapy is an innovative therapeutic option for lung cancer, but only a fraction of the patients experience a favorable response to the treatment, possibly due to factors inherent to the tumor immune microenvironment (TIME). Consequently, the identification of suitable theranostic markers is of paramount importance for the accurate selection of patients who will benefit from ICI therapy [19]. Tobacco smoke is the major cause of lung cancer; specifically, acetaldehyde is one of the prominent carcinogens present in both tobacco smoke and electronic cigarette aerosols [20], which acts inducing DNA damage and inhibiting DNA repair [21]. Concurrent alcohol drinking synergistically further increases the risk for cancers in the upper digestive intestinal tract associated with tobacco smoke [22]. Although the sole role of alcohol consumption in lung cancer is controversial, alcoholic beverages are a well-established source of carcinogens [23]. The lung epithelium is directly exposed to alcohol and its metabolites in the condensation of vapors derived from the bronchial circulation [24], oral cavity, and inhaled air, contributing to the pathophysiology of pulmonary diseases [25]. As a matter of fact, the determination of alcohol in the exhaled breath is implemented in law enforcement; endogenously produced acetaldehyde can also be determined in the breath after alcohol ingestion [26]; moreover, the assessment of aldehydes in breath has been considered as a convenient test for lung cancer [27]. The microbiota is an additional

endogenous source of acetaldehyde. Human lungs are continuously exposed to the microbiome from inhaled air and the upper respiratory tract. Lungs approximately host a dynamic population of 2.2×10^3 bacterial genomes per cm^2, participating in shaping immune tolerance. The presence of colonizing bacteria with ADH activity in the lungs and oral cavity increases the risk of cancer development via the local production of acetaldehyde, which might be significantly elevated in the presence of reduced ALDH2 activity [28]. Moreover, even in the absence of ethanol consumption, exhaled breath may contain acetaldehyde produced through the fermentation of dietary fibers by gut *Faecalibacteria* [29].

ALDH2 is a hot topic of clinical research because the reduced activity of this mitochondrial homo-tetrameric enzyme affects approximately 10% of the global population. ALDH2 dysfunction has been associated with various human disorders, including cardiovascular, neurodegenerative, and liver diseases, diabetes, Fanconi anemia, osteopenia, aging, and different types of cancers [30–32]. Accordingly, ALDH2 may be a promising therapeutic target in numerous diseases [33, 34]. However, relatively little attention has been paid so far to the association between alcohol consumption, pulmonary ALDH2 expression, and lung cancer. In the current study, we explore the potential role of ALDH2 as a theranostic marker for NSCLC.

2 | MATERIALS AND METHODS

2.1 | Expression analysis in healthy tissues

Tissue-specific mRNA and protein expression levels of human ALDH2 were assessed using the European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI) Expression Atlas platform (https://www.ebi.ac.uk/gxa/home). *ALDH2* RNA expression was assessed in the RNA-Seq mRNA baseline data set from the Genotype-Tissue Expression (GTEx) Project [35]; ALDH2 protein expression was evaluated by querying the human proteome The PRoteomics IDEntifications (PRIDE) archive [36] and the EMBL-EBI Expression Atlas platform.

2.2 | Expression analysis in tumor tissues

The expression levels of *ALDH2* in LUAD in comparison with normal samples were analyzed by the Gene Expression Profiling Interactive Analysis (GEPIA) platform (http://gepia.cancer-pku.cn/) [37] on gene expression data from The Cancer Genome Atlas (TCGA). ALDH2 protein expression in lung tumors with different tumor histology was determined by querying the Clinical Proteomic Tumor Analysis Consortium (CPTAC) database by the University of Alabama at Birmingham Cancer Data Analysis Portal (UALCAN) (https://ualcan.path.uab.edu/) [38]. ALDH2 expression analysis at different stages of LUAD was assessed using the GEPIA2 portal (http://gepia2.cancer-pku.cn/) and Gene Set Cancer Analysis (GSCA) platform (http://bioinfo.life.hust.edu.cn/GSCA/#/) [39].

2.3 | Analysis of *ALDH2* expression on cancer-related pathways

The impact of *ALDH2* mRNA expression on different well-defined cancer-related pathways was determined by the GSCA portal, which calculates the activation or inhibition of gene expression in pathway activity groups. The correlation between *ALDH2* expression and onco-suppressor genes expression in the TCGA LUAD cohort was investigated by evaluating the Spearman correlation using the GEPIA2 [37] and cBioPortal for Cancer Genomics (https://www.cbioportal.org/) [40], including mRNA data from 510 patients. Furthermore, the Correlation AnalyzeR web tool (https://gccri.bishop-lab.uthscsa.edu/shiny/correlation-analyzer/) [41] was employed to analyze the correlation between *ALDH2* and selected biological pathways by correlation-based Gene Set Enrichment Analysis (corGSEA).

2.4 | Association of gene expression with *TP53* mutation status

The association of *ALDH2* expression with *TP53* mutation status in LUAD was investigated using the UALCAN portal, which uses *TP53* mutational status obtained from the TCGA whole-exome sequencing data from the Genomic Data Commons portal. The samples with or without *TP53* mutations were matched with TCGA RNA-seq expression data.

2.5 | Analysis of DNA methylation

Promoter methylation levels of *ALDH2* were assessed using the UALCAN platform on DNA methylation and gene expression data from TCGA.

2.6 | Survival analysis

Univariate survival analyses were performed to estimate the hazard ratios (HRs) with 95% confidence intervals

(CIs) and Kaplan–Meier survival plots were generated using the Tumor online Prognostic analyses Platform (ToPP) (http://www.biostatistics.online/topp/index.php) [42], which collects multi-omics and clinical data from TCGA, International Cancer Genome Consortium and the CPTAC project.

2.7 | Analysis of ALDH2 expression at the single-cell level

Expression of ALDH2 at the single-cell level in normal pulmonary tissue was analyzed using the Human Protein Atlas (HPA) (https://www.proteinatlas.org/humanproteome/single+cell+type) [43] and the GTEx Portal (https://gtexportal.org/home/); analysis in the LUAD TIME was performed using the Tumor Immune Single-cell Hub 2 (TISCH2) (http://tisch.comp-genomics.org/home/) on the publicly available scRNA-seq data set from single-cell transcriptomic analyses of cells isolated from NSCLC (NSCLC_GSE131907).

2.8 | Analysis of ALDH2 expression and tumor–immune system interactions

The relations between tumor and immune system interactions were analyzed using the TISIDB (http://cis.hku.hk/TISIDB/index.php) [44] web portal. In particular, the relative abundance of tumor-infiltrating lymphocytes (TILs), according to previously described immune-related signatures of 28 TIL types [45], and ALDH2 expression in LUAD, was inferred by using gene set variation analysis based on gene expression profile.

3 | RESULTS

3.1 | ALDH2 is expressed in the healthy lungs

To investigate the potential role of ALDH2 as a therapeutic target in lung cancer, we explored the basal protein and mRNA expression levels in healthy lung tissue by employing the European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI) Expression Atlas and the GTEx Project repository, respectively. Both proteomic and transcriptomic data in selected healthy human tissues indicate that ALDH2 is highly expressed in the liver and other tissues with prominent mitochondrial function, including the heart, kidney, and lungs (Figure 1a,b). Most of the ingested alcohol is normally metabolized in the liver, but also other tissues, including the gastrointestinal

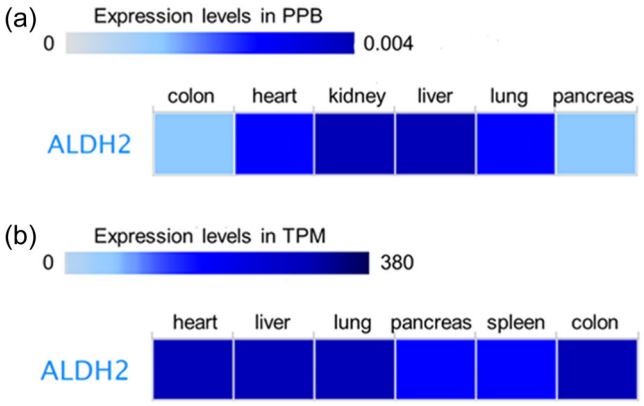

FIGURE 1 Tissue-specific expression of ALDH2. ALDH2 expression levels analyzed by using the European Molecular Biology Laboratory-European Bioinformatics Institute Expression Atlas platform. (a) Protein expression levels from the human proteome data set; (b) RNA expression levels from the Genotype-Tissue Expression project data set. ALDH2, aldehyde dehydrogenase 2; PPB, parts per billion; TPM, transcripts per million.

mucosa, spleen, and lungs, can metabolize ethanol [46]. Furthermore, ALDH2 is one of the most expressed ALDH isoforms in the lungs, where it detoxifies acetaldehyde absorbed from the bronchial circulation and inhaled from exogenous sources [47]. Expression of other members of the ALDH enzyme superfamily, including ALDH1A1 and ALDH3A1, is altered in lung cancer [48], but ALDH isozymes exhibit distinct substrate specificity and pathophysiology [14].

3.2 | ALDH2 expression is reduced in lung cancer

There is no consensus on the association between alcohol drinking and lung cancer [49]; nonetheless, the incidence of lung and alcohol-associated cancers is higher in approximately 40% of the East Asian population, characterized by the prevalence of the *ALDH2* inactivating polymorphism rs671 (ALDH2*2) [50, 51] that leads to acetaldehyde accumulation. Acetaldehyde, however, is not only a metabolite of ethanol but is also present in tobacco and e-cigarette smoke; accordingly, smokers with the ALDH2*2 polymorphism have an increased risk of lung cancer [52, 53].

Transcriptional analysis of TCGA data and proteomic analysis of the CPTAC data indicate that ALDH2 expression in LUAD is lower than in normal lung tissue (Figure 2a,b) [54–56], regardless of tumor stage (Figure 3). Nevertheless, as shown in Figure 3, a significant albeit slight reduction in ALDH2 expression can be observed between Stages I and IV patients, suggesting that a lower level of the enzyme is

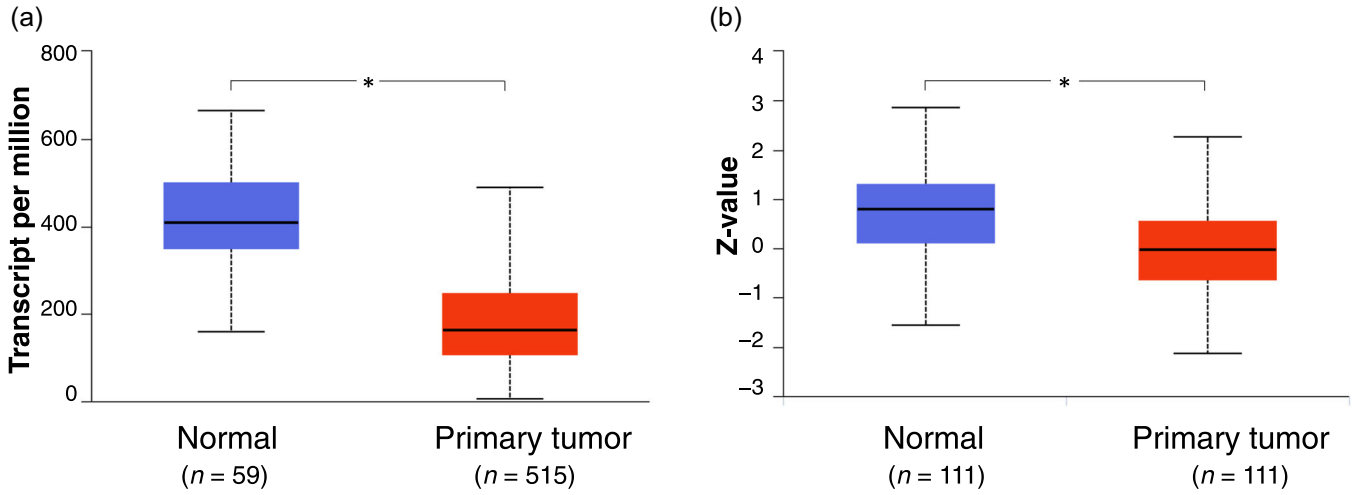

FIGURE 2 Gene expression profiling of ALDH2 in lung adenocarcinoma (LUAD). (a) The expression levels of *ALDH2* in tumor tissues (red) and normal samples (blue) in gene expression data from The Cancer Genome Atlas. (b) Protein expression of ALDH2 in LUAD querying the Clinical Proteomic Tumor Analysis Consortium database. Analyses were performed by the University of Alabama at Birmingham Cancer Data Analysis Portal. The asterisk (*) indicates a statistically significant difference between the indicated groups ($p \leq 0.05$). ALDH2, aldehyde dehydrogenase 2.

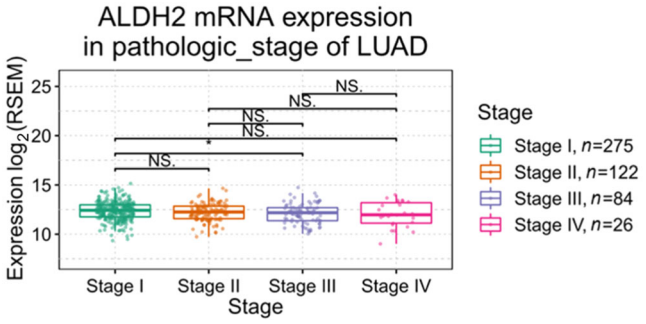

FIGURE 3 *ALDH2* expression in different stages of lung adenocarcinoma (LUAD). The pathological stage plot created by using the Gene Set Cancer Analysis platform on TCGA data to compare *ALDH2* expression in various stages of LUAD. TCGA, The Cancer Genome Atlas. The asterisk (*) indicates a statistically significant difference between the indicated groups ($p \leq 0.05$). NS. indicates not significant.

indicative of a worse prognosis. Interestingly, *ALDH2* mRNA expression is higher in LUAD non-smoker patients than in smokers, who are subjected to increased acetaldehyde exposure (Figure S1).

Using the Expression and Pathway Activity section in the GSCA platform, we assessed the possible effects of altered *ALDH2* mRNA expression on cancer-related pathways in the TCGA LUAD data sets including 517 tumor and 59 normal samples. The analysis indicates that higher *ALDH2* expression exerts potential inhibitory effects on cancer-related activity associated with apoptosis, cell cycle, and epithelial–mesenchymal transition (EMT) pathways (Figure 4).

Collectively, these data suggest that the ALDH2 isoform might impact tumor-suppressive pathways in LUAD.

3.3 | *ALDH2* expression is reduced in *TP53*-mutant lung cancer compared to non-mutant

Tumor suppressor protein TP53 dysregulation has been associated with many cancers, including lung cancer. Mutations in the *TP53* gene generally give rise to increased stability and accumulation of the genetic product and occur in up to 46% of LUAD and 82% of LUSC patients; patients with *TP53*-mutated NSCLC generally have worse prognoses and are resistant to current therapies [57]. Both alcohol drinking and cigarette smoking are associated with *TP53* mutations in NSCLC [58]. To investigate a potential correlation between ALDH2 and TP53 status in lung cancer patients, we examined the level of expression of *ALDH2* in the cohort of *TP53*-mutated NSCLC. *TP53* mutation status was obtained from the TCGA whole-exome sequencing data and mutation annotation from VarScan2 was obtained from the National Cancer Institute Genomic Data Commons (CRDC) portal. Analysis of the relative expression of *ALDH2* in normal healthy tissue or LUAD with different *TP53* mutation statuses by the UALCAN platform indicates that *ALDH2* expression in *TP53*-mutant LUAD is lower than that in non-*TP53*-mutant tumors (Figure 5). Recently, several studies indicated that *TP53* mutations are associated with improved survival in patients treated with immune checkpoint blockade therapy [59]. Therefore, it could be suggested that lower *ALDH2* expression associated with *TP53* mutations could be a useful predictive factor to identify LUAD patients who might benefit from ICI treatment.

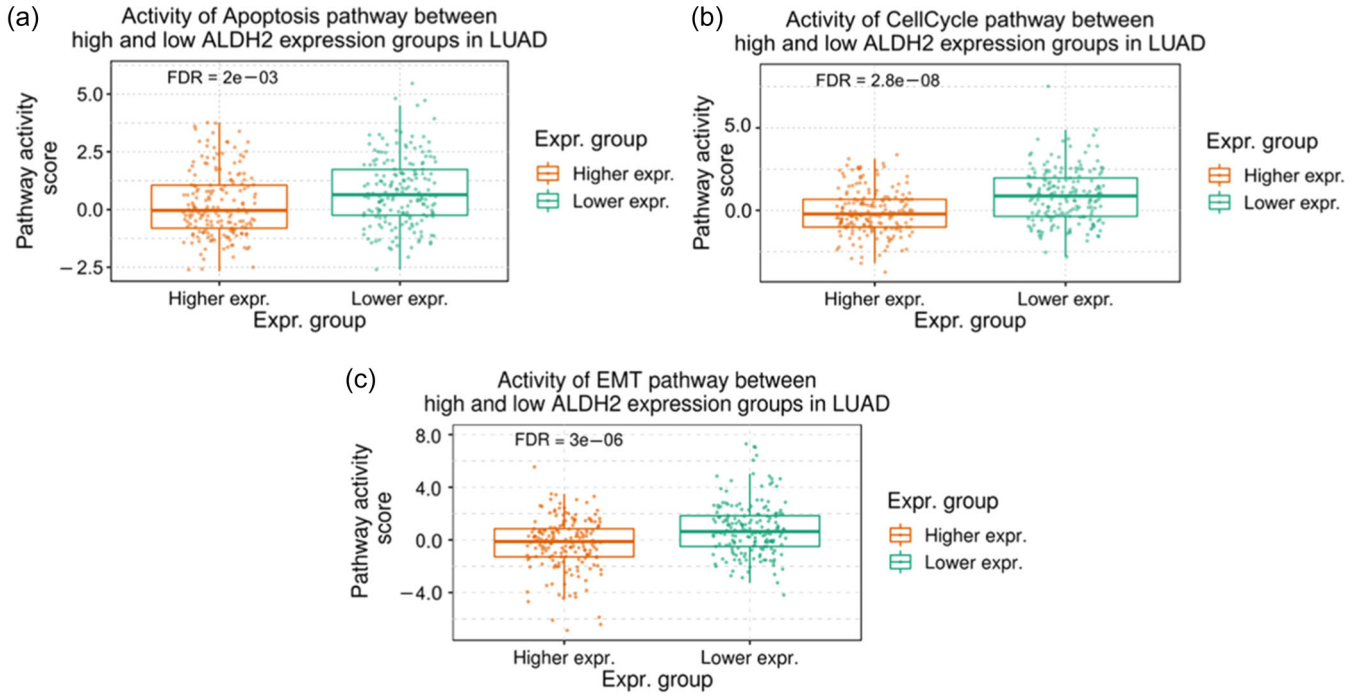

FIGURE 4 Differences of pathway activity between high and low *ALDH2* mRNA expression. GSCA analysis of the effects of *ALDH2* expression on the activity of (a) apoptosis, (b) cell cycle, and (c) epithelial–mesenchymal transition (EMT) pathways in LUAD. GSCA, Gene Set Cancer Analysis.

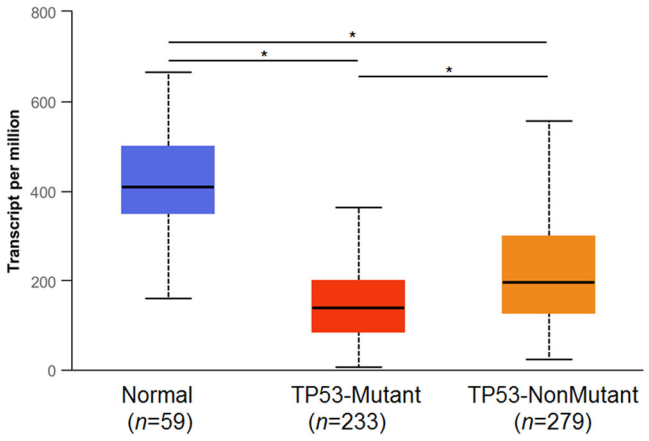

FIGURE 5 *ALDH2* expression analysis in lung adenocarcinoma based on *TP53* mutation status. The UALCAN platform was used to analyze the relative expression of *ALDH2* in normal healthy tissue or lung cancers with different *TP53* mutation statuses. The samples with/without *TP53* mutation were matched with RNA-seq TCGA data. TCGA, The Cancer Genome Atlas; UALCAN, University of Alabama at Birmingham Cancer Data Analysis Portal. *$p \leq 0.05$.

3.4 | *ALDH2* promoter methylation is reduced in lung cancer

Global and gene-specific promoter aberrant DNA methylation have been extensively described in lung cancer [60], and the transcriptional response may be context-specific [61]. Recently, altered methylation pattern of *ALDH2* has been described in different types of cancer [31, 56], including LUAD [54], where epigenetic silencing induced by methylation may facilitate bone metastasis [62]; reduced levels of *ALDH2* DNA methylation have been observed in gastric tumors, but the impact on gene expression in the context of extremely low levels of DNA methylation has not been clearly determined [63]. On these bases, we verified the DNA methylation status in the *ALDH2* promoter in LUAD in data sets from TCGA using the UALCAN platform (Figure 6). Differential *ALDH2* DNA methylation patterns were detected in LUAD patients in comparison to healthy controls; even though the analysis of the beta value does not reflect a transition from hyper-methylation to hypo-methylation, a significant reduction in the methylation levels in the promoter of the *ALDH2* gene was assessed in LUAD data sets.

3.5 | *ALDH2* expression positively correlates with the onco-suppressor genes selenium-binding protein 1, folate receptor alpha, and lactate dehydrogenase D

Using the cBioPortal, we investigated the genes whose expression is correlated to *ALDH2* in the TCGA

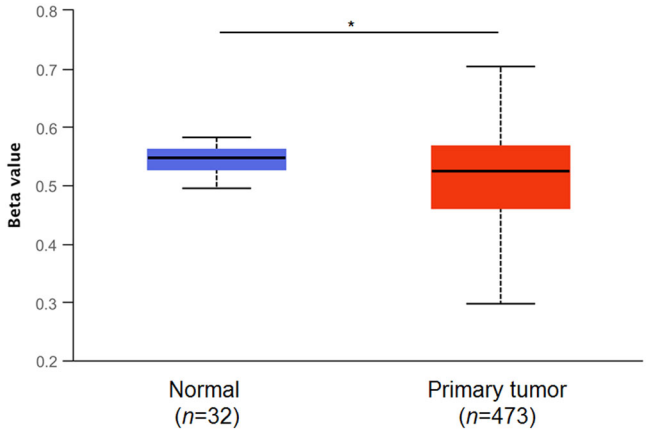

FIGURE 6 *ALDH2* methylation analysis in lung adenocarcinoma. Promoter methylation levels of *ALDH2* assessed using the UALCAN platform on TCGA samples; the beta value indicates the level of DNA methylation ranging from 0 (unmethylated) to 1 (fully methylated). Different beta value cutoffs have been considered to indicate hyper-methylation [beta value: 0.70–0.50] or hypo-methylation [Beta value: 0.30–0.25]. TCGA, The Cancer Genome Atlas; UALCAN, University of Alabama at Birmingham Cancer Data Analysis Portal. *$p \leq 0.05$.

Firehose Legacy LUAD cohort including mRNA data from 230 patients. Spearman correlation was utilized to evaluate the statistical significance. Interestingly, dysfunctional expression of the genes with higher correlation coefficients with *ALDH2* expression has been previously associated with NSCLC. In particular, the genes with the highest positive correlation coefficients were: selenium-binding protein 1 (*SELENBP1*) (Spearman's Rank correlation coefficient—ρ: 0.65) [64]; folate receptor alpha (*FOLR1*) (ρ: 0.57) [65]; lactate dehydrogenase D (*LDHD*) (ρ: 0.54) [66], while top 3 genes with higher negative correlation coefficients were: discs large homolog associated protein 5 (*DLGAP5*) (ρ: −0.56) [67]; anillin (*ANLN*) (ρ: −0.56) [68]; and cytoskeleton-associated protein 2 like (*CKAP2L*) (ρ: −0.55) [69], as illustrated in Figure 7. Additional genes correlated with *ALDH2* expression in LUAD are reported in Table S1.

Moreover, we used the Correlation AnalyzeR web tool (https://gccri.bishop-lab.uthscsa.edu/shiny/correlation-analyzer/) [41] to obtain co-expression correlation for *ALDH2* and selected biological pathways by corGSEA. Interestingly, top-ranking pathways

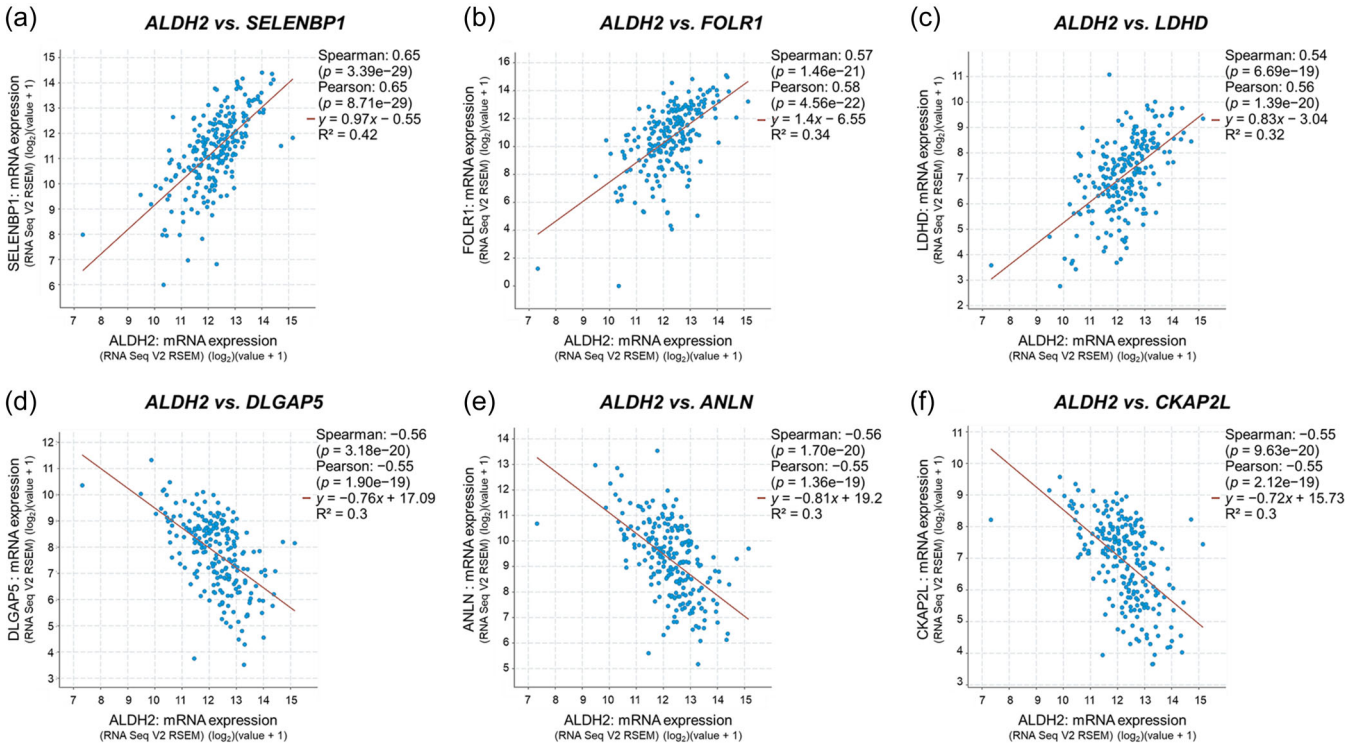

FIGURE 7 *ALDH2* versus selected gene expression correlation analysis in LUAD. Analysis was performed using the cBioPortal for Cancer Genomics, TCGA Firehose Legacy LUAD data set. (a) *SELENBP1*: selenium-binding protein 1; (b) *FOLR1*: folate receptor alpha; (c) *LDHD*: lactate dehydrogenase D; (d) *DLGAP5*: discs large homolog-associated protein 5; (e) *ANLN* anillin; and (f) *CKAP2L*: cytoskeleton-associated protein 2 like. LUAD, lung adenocarcinoma; TCGA, The Cancer Genome Atlas; TPM, transcripts per million reads.

correlating with *ALDH2* expression in lung cancer include the gene ontology (GO) terms antigen binding, humoral immune response mediated by circulating immunoglobulin, and regulation of humoral immune response (Figure S2) suggesting that ALDH2 might be involved in the modulation of immune response.

3.6 | ALDH2 expression has a prognostic value in LUAD

We examined the significance of the survival difference in LUAD patients defined subgroups with different levels of ALDH2 expression. To assess patient prognosis, Kaplan–Meier survival plots were generated using ToPP. According to this analysis, higher ALDH2 expression is predictive of improved overall survival (OS) in LUAD patients; moreover, an increase in the proportion of patients diagnosed with stage I LUAD can be observed in the cohort of subjects with higher ALDH2 expression (Figure 8). Similar results were obtained assessing progression-free interval (PFI), disease-specific survival (DSS), disease-free interval (DFI), and relapse-free survival (RFS) (Figure S3).

3.7 | ALDH2 expression and immune cell infiltration in LUAD tumor microenvironment

Analysis of the single-cell expression data sets accessible through the HPA (Figure 9a) and the GTEx (not shown) portals indicate that in healthy lung tissue mainly alveolar epithelial cells and macrophages express ALDH2. Accordingly, *ALDH2* is part of the "Monocytes—Inflammatory response cluster" identified by the HPA portal on the basis of RNA expression data across different tissues. We also analyzed *ALDH2* expression in the TIME using the TISCH2 platform in the single-cell transcriptomic NSCLC_GSE131907 data set. Within the LUAD TIME, the strongest *ALDH2* expression is associated with the monocyte/macrophage compartment and to a lesser extent to cancer-associated cells, alveolar, and endothelial cells (Figures 9b,c). These data are in accordance with single-cell RNA-Seq analysis performed in different cancer types, indicating that within the tumor microenvironment, *ALDH2* expression is mostly enriched in macrophages, monocytes, and cancer-associated fibroblasts [70, 71].

To further explore the potential role of *ALDH2* in modulating immune cell compartment, we assessed the correlation coefficient between *ALDH2* expression and immune cell infiltration in the LUAD tumor microenvironment using the GSCA platform (Figure 10a). In particular, *ALDH2* expression was positively correlated with mucosal-associated invariant T (MAIT) (ρ: 0.48) and T helper 17 (Th17) (ρ: 0.25) cell scores; conversely, a negative correlation was determined with exhausted T cell (ρ: −0.32) and naturally occurring regulatory T cells (nTreg) (ρ: −0.50) (Figure 10a). Scatter plots in panels (b–e) in Figure 10 indicate the Spearman correlation between *ALDH2* expression and MAIT, Th17, exhausted T cell, and nTreg, respectively.

A recent study deeply investigated the role of ALDH2 in cancer [56], revealing a significant association between ALDH2 and several immune

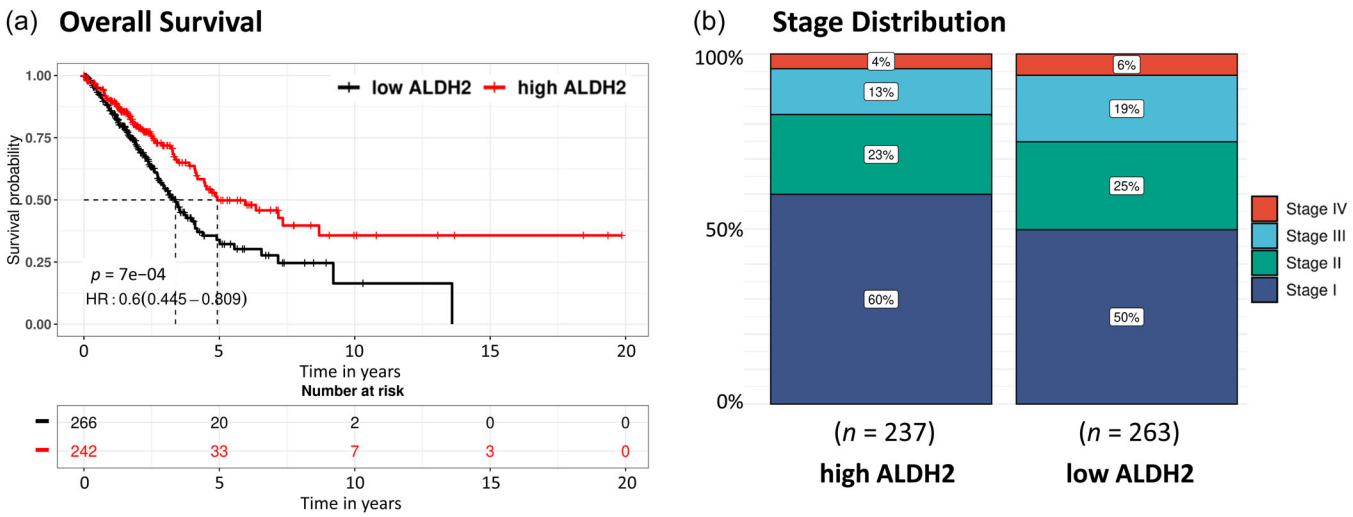

FIGURE 8 Prognostic value of ALDH2 in lung adenocarcinoma (LUAD). Higher ALDH2 expression is related to (a) increased overall survival and (b) increased number of patients at Stage I. Analysis was performed using ToPP on TCGA LUAD data. TCGA, The Cancer Genome Atlas; ToPP, Tumor online Prognostic analyses Platform.

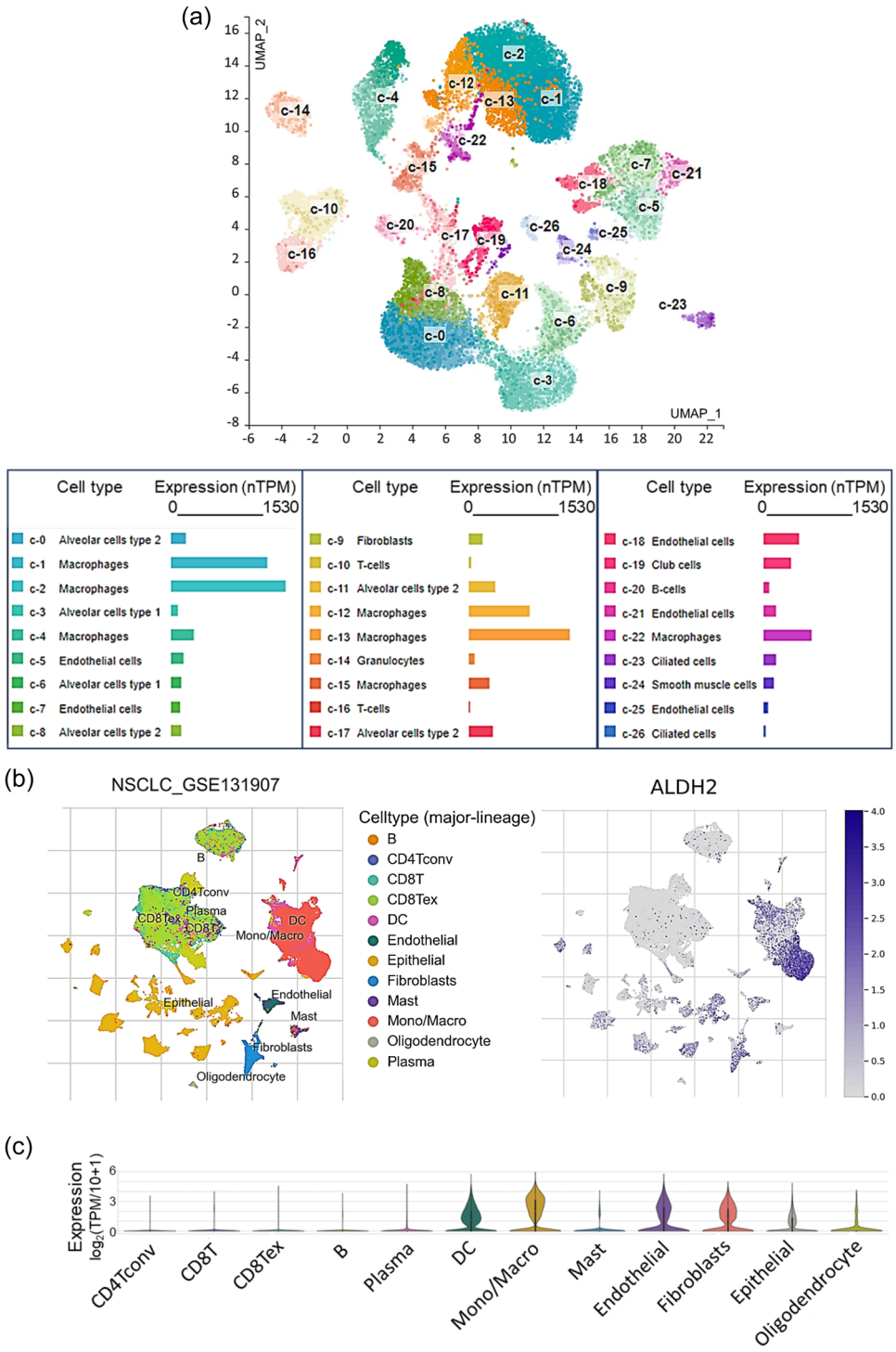

FIGURE 9 *ALDH2* expression of at the single-cell level in the tumor microenvironment. (a) Uniform manifold approximation and projection (UMAP) plot showing single-cell RNA-seq analysis in healthy lung tissue according to the HPA platform. Expression levels indicated in normalized transcripts per million (range 0–1550). (b) UMAP and (c) violin plots showing the distribution of *ALDH2* expression in different cell types in the single-cell transcriptomic non-small cell lung cancer data set NSCLC_GSE131907. Analysis was performed with the TISCH2 platform.

populations, including $CD4^+$ T cells, $CD8^+$ T cells, B cells, neutrophils, and macrophages, in different tumor types. This evidence strongly corroborates the prognostic role of ALDH2 in cancer, with a consistent correlation with the tumor immune microenvironment.

3.8 | *ALDH2* expression levels correlate with the expression of immune inhibitors and immune stimulators

Using the TISIDB platform, we assessed the correlation between *ALDH2* expression in LUAD and the level of

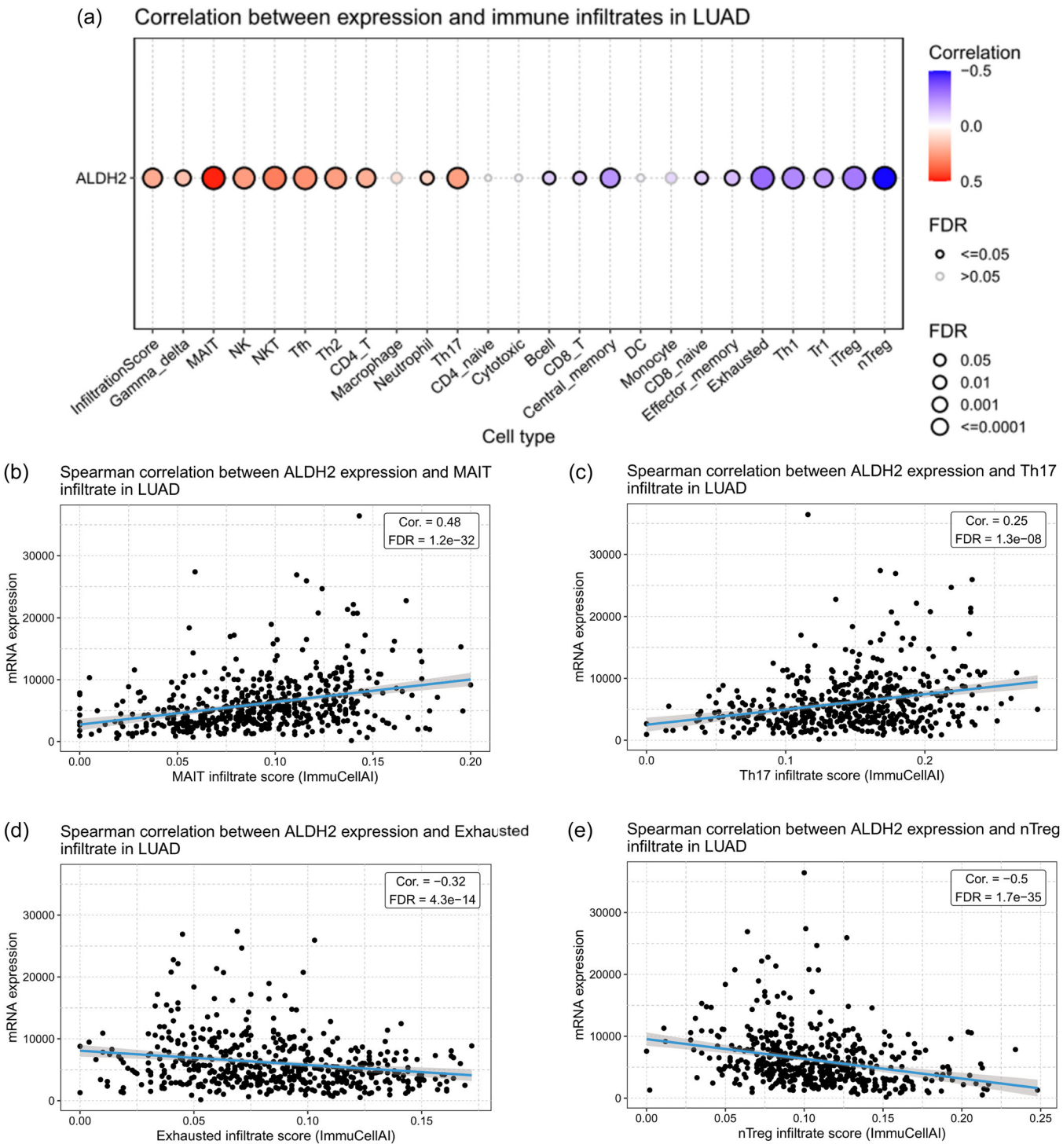

FIGURE 10 Correlation analysis between ALDH2 and immune infiltrate in lung adenocarcinoma. (a) Correlation between *ALDH2* expression and immune cell infiltration in the tumor microenvironment of LUAD. The color indicated Spearman's correlation coefficient and the circle size represented the false discovery rate (FDR) value; the smaller the value, the larger the circle. Graphs showing the Spearman correlation between *ALDH2* expression and infiltration scores of (b) mucosal-associated invariant T (MAIT) cells, (c) T helper 17 (Th17) cells, (d) exhausted T cell, and (e) naturally occurring regulatory T (nTreg) cells. Analysis was performed using the GSCA platform. GSCA, Gene Set Cancer Analysis; LUAD, lung adenocarcinoma.

immune inhibitors by calculating the Spearman's correlation coefficients (Figure 11). The analysis revealed a significant inverse correlation between *ALDH2* expression and the levels of major immune inhibitors such as cytotoxic T-lymphocyte antigen 4 (*CTLA-4*) (ρ: −0.11), indoleamine 2,3-dioxygenase 1 (*IDO1*) (ρ: −0.13), lymphocyte-activation gene 3 (*LAG3*) (ρ: −0.16), micro (*PD-1*, *PDCD1*) (ρ: −0.15), programmed cell death ligand

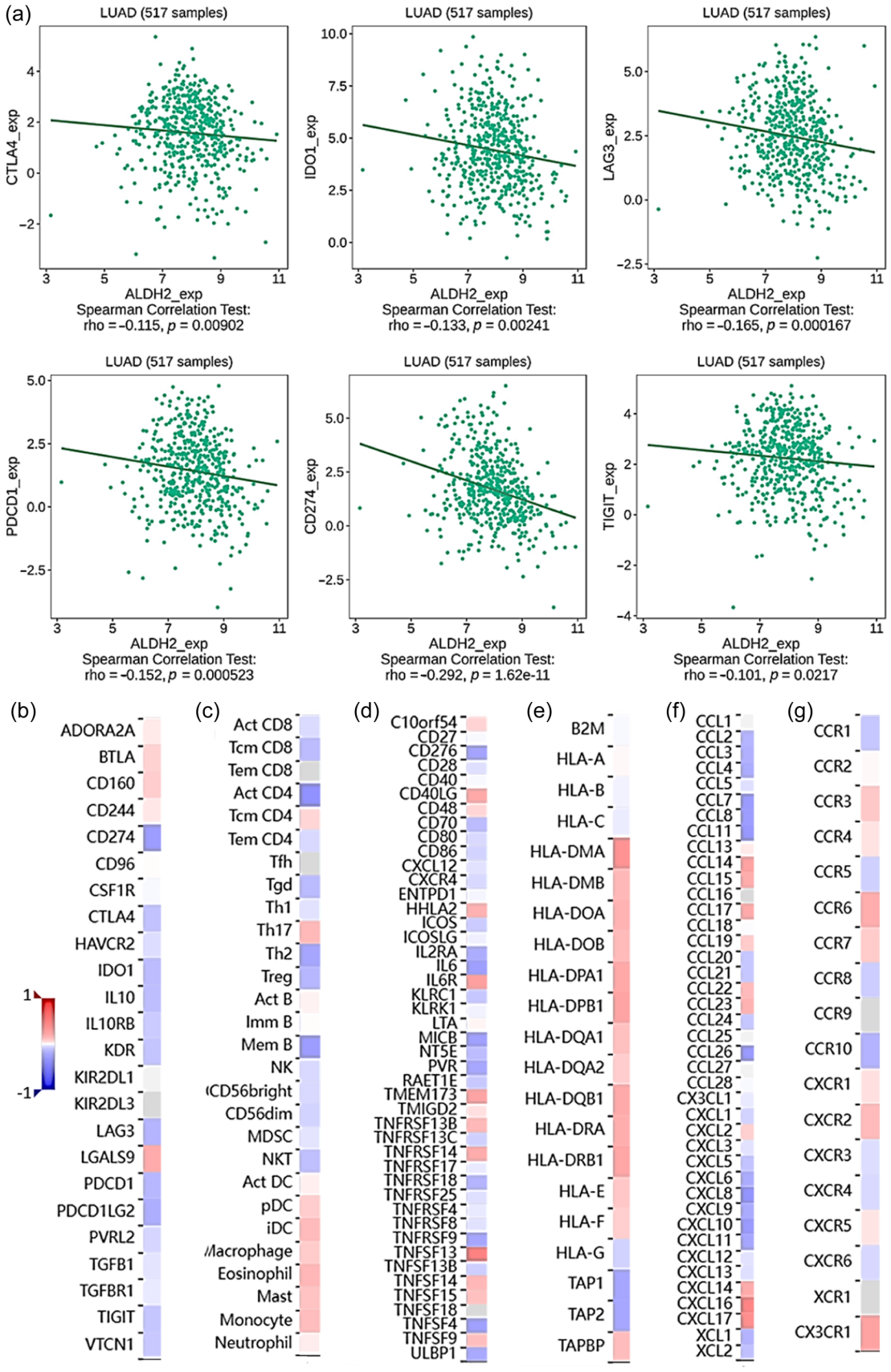

FIGURE 11 (See caption on next page).

1 (*PD-L1*, *CD274*) (ρ: −0.29), and T cell immunoreceptor with Ig and ITIM domains (*TIGIT*) (ρ: −0.10) (Figures 11a,b) suggesting that LUAD patients with high ALDH2 expression might not be considered as appropriate candidates to receive ICI therapy. Moreover, using the TISIDB platform, we investigated the potential correlation between *ALDH2* expression in LUAD and the levels of immune stimulators, major histocompatibility complex (MHC) molecules, chemokines, and chemokine receptors. Results are shown as representative heatmaps in Figure 11c–g, respectively.

Collectively, these data suggest that ALDH2 might be involved in the modulation of immune response and the recruitment of immune-infiltrating cells in LUAD.

4 | DISCUSSION

ALDH2 plays a pivotal role in detoxification of aldehydes and redox homeostasis. The potential involvement of ALDH2 in several types of cancers, including digestive system, breast, liver, and lung cancers, and head-neck squamous cell carcinoma, has been extensively described [31, 34, 56]. In particular, reduction of ALDH2 activity linked to the ADLH2*2 genotype has been associated with upper aerodigestive and digestive and cancers [51, 72–77]. Depending on the type of cancer, ALDH2 can act as pro-oncogenic, promoting cell survival of cancer cells, or anti-oncogenic, reducing the toxic effect of aldehydes [72, 73]. ALDH2 overexpression can, in fact, promote cancer progression and resistance to chemotherapy in clear-cell renal cell carcinomas and bladder cancer [74, 75], whereas ALDH2 inhibition suppresses tumor growth, increasing the infiltration of $CD3^+$ and $CD8^+$ T lymphocytes in a mouse model of colorectal cancer [76]. Recently, it has been demonstrated that ALDH2 is downregulated in HNSC [55], gastric cancer [77], and melanoma [78] compared with healthy tissues and that, in affected subjects, higher ALDH2 expression is associated with a better prognosis. Notably, reduction of ALDH2 expression observed in tumor tissue results in an increase of acetaldehyde that promotes collagen production in human fibroblasts [79], a hallmark of solid tumors [80], remodeling tumor ECM and affecting immune surveillance. In addition to acetaldehyde, ALDH2 detoxifies other reactive aldehydes, including methylglyoxal, a glucose-derived precursor of advanced glycation end-products (AGEs). Methylglyoxal is a potent immunosuppressor and inhibitor of T cell function; therefore, enhancing its detoxification may improve the efficacy of ICI therapy [81]. Since ALDH2 plays an important role in the development of specific cancers, targeting ALDH2 may provide a new putative strategy for cancer therapy [34].

In the present study, we focused our attention on the specific association between ALDH2 expression and LUAD, and, by using publicly available data sets, we observed that ALDH2 expression in LUAD is lower than in normal lung tissue, in line with data recently described for HNSC and LUSC [55]. Based on the demonstration that low *ALDH2* expression levels, associated with high *XRCC1* expression, are predictive of poor prognosis and low OS in patients with lung and liver cancers [82], we specifically evaluated the association between ALDH2 expression and survival in LUAD patients. In particular, using ToPP, we generated Kaplan–Meier survival plots showing that higher ALDH2 expression was predictive of improved OS, with a higher expression in stage I-LUAD patients. Searching for specific cancer-related pathways that could be differently modulated according to ALDH2 expression, we used the GSCA platform, evidencing the inhibitory effect of ALDH2 expression on cancer-related activity, in particular with those associated with cell cycle, apoptosis, and EMT pathways, thus mainly impacting tumor suppressive pathways in LUAD. We further assessed the role of ALDH2 in LUAD in dependence of the *TP53* mutation status, taking advantage of the UALCAN platform. The analysis evidenced that ALDH2 expression was reduced in *TP53*-mutant lung cancer compared with nonmutant. As recent studies indicated that *TP53* mutations could be associated with improved survival in patients treated with ICI therapy [59], we speculated the potential use of the associated lower ALDH2 expression as a predictive factor to identify LUAD patients who might benefit from ICI treatment. Furthermore, correlation analysis revealed that top genes positively correlated with *ALDH2* expression in LUAD act as onco-suppressor genes, whereas negatively correlating genes are mainly oncogenes. In particular, selenium-binding protein 1 (*SELENBP1*),

FIGURE 11 Correlation between *ALDH2* expression and immune infiltrates. (a) Correlation analysis between *ALDH2* expression and *CTLA-4*, *IDO1*, *LAG3*, *PD-1*, *PD-L1* (*CD274*), and *TIGIT* levels. Heatmaps showing relationship between *ALDH2* expression and (b) immunoinhibitors, (c) abundance of 28 tumor-infiltrating lymphocytes (TILs) signature, (d) immunostimulators, (e) MHC molecules, (f) chemokine, and (g) chemokine receptors in LUAD. Analysis was performed using the TISIDB platform. LUAD, lung adenocarcinoma; MHC, major histocompatibility complex.

which is a tumor suppressor in NSCLC [64], is the most strongly correlated gene with ALDH2 expression in LUAD. Similarly, high expression of folate receptor alpha (*FOLR1*) [65] and lactate dehydrogenase D (*LDHD*) [83] exert a protective effect in LUAD. Conversely, cytoskeleton associated protein 2 like (*CKAP2L*), the top inversely correlated gene with *ALDH2* expression, acts as an oncogene in LUAD [69]; anillin is also overexpressed in LUAD [68] and associates with metastasis [84]. Likewise, the high expression of the discs large homolog associated protein 5 (*DLGAP5*) indicates poor prognosis and response to immunotherapy in LUAD [67].

Interestingly, single-cell RNA-Seq analysis performed for different cancer types highlighted that, within the tumor microenvironment, *ALDH2* expression is mostly enriched in macrophages, monocytes, and cancer-associated fibroblasts [70, 71]. Accordingly, using the HPA, we observed that in healthy lung tissue mainly macrophages express ALDH2, while the TISCH2 platform (in the single-cell transcriptomic NSCLC_GSE131907 data set) showed that, in LUAD, *ALDH2* is strongly expressed in the monocyte/macrophage compartment and to a lesser extent to cancer-associated cells, alveolar, and endothelial cells. The existence of a direct association between immune infiltration and ALDH2 has been demonstrated in a recent study in HNSC [55], showing that ALDH2 expression correlates with immune marker genes and T cell infiltration. In particular, ALDH2-mediated aldehyde metabolism promotes tumor immune evasion by activating the NOD/NF-κB/VISTA axis [85]. On these bases, we interrogated the GSCA platform to assess a possible correlation between ALDH2 and immune cell infiltration in LUAD and observed that ALDH2 expression correlates with mucosal-associated invariant T cell (MAIT) infiltration. The increased frequency of MAIT cells has recently been implicated in dysfunctional immune response in lung cancer [86] and has been suggested as a predictor of anti-PD-1 immunotherapy response [87]. Moreover, ALDH2 expression is correlated with infiltration of Th17 cells, which are an important constituent of the inflammatory milieu of the lung tumor immune microenvironment (TIME) [88]. Conversely, we determined a negative correlation between ALDH2 expression and exhausted T cells and naturally occurring regulatory T cells (nTreg) that play a role in remodeling the immune-suppressive pulmonary TIME. In particular, in lung cancer, T-cell exhaustion is associated with decreased cytokine production and cytolytic activity, and increased expression of ICI receptors, leading to the failure of cancer eradication by ICI therapy [89]. Also, infiltration of nTregs, an immunosuppressive subset of $CD4^+$ T cells, has a negative prognostic value for patients with NSCLC and impacts the efficacy of ICI therapy [90, 91].

ICI therapy is an innovative therapeutic option for lung cancer, but only a fraction of patients experience a favorable response to the treatment; therefore, an accurate patient selection is of paramount importance to predict patients who might benefit from ICI therapy. However, patients initially responsive to ICI therapy may develop resistance. ALDHs may represent a target to potentially overcome anticancer therapy resistance [92, 93]. Therefore, unveiling the role of ALDH2 in lung tumor progression and immune infiltration could prove the potential use of ALDH2 as a predicting marker for immunotherapeutic response in lung cancer. A correlation between ALDH2 and immune inhibitors has recently emerged [56], thus suggesting a role for ALDH2 as a prognostic and diagnostic biomarker to predict the sensitivity of HNSC patients to ICI therapy [94], as well as in hepatocellular carcinoma [95], and glioma [71]. Based on multi-omics data analysis in different cancers, ALDH2 has also been suggested as an accurate biomarker with the best prediction efficacy in evaluating immunotherapeutic response in skin cutaneous melanoma, compared with the most commonly used biomarkers (PD-1, PD-L1, CTLA4, CD8, and TMB) [31]. These findings shed new light on the potential role of ALDH2 in more precisely tailoring cancer immunotherapy, predicting patients who might benefit from ICI therapy. Interestingly, in hepatocellular carcinoma, high ALDH2 expression correlates with poor dendritic cells and macrophages immune infiltration and with low PD-1 and CTLA4 expression [95]. Clinical results also indicate that ALDH2 is among the mitochondrial metabolic proteins significantly reduced in the group of nonresponder melanoma patients undergoing anti-PD1 immunotherapy [96] and correlates to immune cell infiltration [78]. Accordingly, *ALDH2* polymorphism rs671, which reduces the ALDH2 enzymatic activity, has been proposed as an easily detectable predictor of the efficacy of PD-1/PD-L1 inhibitor treatment in patients with lung cancer [97]. To examine whether ALDH2 might affect NSCLC immunotherapy, we performed a comprehensive analysis based on publicly available multi-omic data. We revealed a significant inverse correlation between *ALDH2* expression and the levels of some immune inhibitors such as cytotoxic T-lymphocyte antigen 4 (*CTLA-4*), indoleamine 2,3-dioxygenase 1 (*IDO1*), lymphocyte-activation gene 3 (*LAG-3*), programmed cell death protein 1 (*PD-1*), programmed cell death ligand 1 (*PD-L1*), and T cell immunoreceptor with Ig and ITIM domains (*TIGIT*). Conversely, *ALDH2* expression is positively correlated with some immune stimulators such as *IL6R* (ρ: 0.27), whose low expression predicts poor LUAD prognosis [98], and negatively correlated with *CD276* (ρ: −0.25) that is highly expressed and impacts the

survival of NSCLC patients [99]. In addition, ALDH2 correlates with the MHC Class II, DM Alpha (*HLA-DMA*) (ρ: 0.35), which is a favorable prognostic marker, and inversely correlates with *TAP1* (ρ: −0.24), whose expression negatively impacts survival in LUAD patients [100]. Finally, we determined that *ALDH2* expression correlates with the CXC chemokine family members *CXCL16* (ρ: 0.45) and *CXCL17* (ρ: 0.39), which exert anti-tumors effects [101], and with the C-C motif chemokine receptor 6 (*CCR6*) (ρ: 0.20), which is a marker of favorable LUAD prognosis [102].

5 | CONCLUSION

Our study, based on the bioinformatics analysis of data available in public databases, supports the use of ALDH2 a potential molecular biomarker for the prognosis and treatment of LUAD. In particular, ALDH2 impacts the immune cell compartment, potentially influencing the efficacy of ICI therapy for pulmonary adenocarcinoma. However, experimental and clinical analyses are needed to verify these results and to identify the molecular mechanisms by which ALDH2 might be instrumental in the selection of the patients who might benefit from immunotherapy.

AUTHOR CONTRIBUTIONS

Silvia Baldari: Conceptualization (equal); data curation (equal); investigation (equal); writing—original draft (equal); writing—review and editing (equal). **Annalisa Antonini**: Investigation (supporting); methodology (equal). **Giuliana Di Rocco**: Writing—review and editing (equal). **Gabriele Toietta**: Conceptualization (equal); data curation (equal); funding acquisition (lead); investigation (equal); writing—original draft (lead); writing—review and editing (lead).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

ETHICS STATEMENT

Not applicable.

INFORMED CONSENT

Not applicable.

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SUPPORTING INFORMATION

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