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Aldehyde dehydrogenase 1B1 is a potential marker of colorectal tumors

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Summary. Colorectal cancer (CRC) is common worldwide. Aldehyde dehydrogenase 1B1 (ALDH1B1), a member of the ALDH1 family, serves as a biomarker for cancer stem cells. We hypothesized that ALDH1B1 expression is associated with colorectal tumors. Immunohistochemistry was used to detect ALDH1B1 expression across a commercial colorectal tissue microarray. The signal intensities of the positively stained tissues were expressed using the mean integrated optical density (mean IOD). We also analyzed *ALDH1B1* mRNA expression in the Oncomine database. The associations between ALDH1B1 expression and CRC stage and prognosis were then evaluated using the web-based tools, GEPIA and UALCAN. Analysis of the tissue microarray revealed that the expression of ALDH1B1 was significantly higher in colorectal adenomas and colorectal adenocarcinoma (IOD/area values = 0.117±0.070 and 0.168±0.0168, respectively) compared with normal and cancer-adjacent tissues (IOD/area values = 0.051±0.028 and 0.068±0.053). For samples collected in the hospital, ALDH1B1 was highly expressed in the adenoma (IOD/area = 0.103±0.054) and CRC (IOD/area = 0.116±0.059) tissues compared with the cancer-adjacent tissues (IOD/area = 0.066±0.024, $p<0.05$). The expression of ALDH1B1 in tissues from two resources was not found to be significantly associated with CRC stage. In Oncomine, *ALDH1B1* mRNA expression was increased in the colorectal tumor tissues compared with the normal colorectal tissues ($p=0.024$) and its expression was independent of CRC stage and prognosis ($p<0.05$). Thus, while the protein and mRNA expression of ALDH1B1 suggests that it is a potential marker of colorectal tumors, its expression is independent of CRC stage and prognosis.

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Introduction

According to estimates published in 2015 by the World Health Organization, cancer is the first or second leading cause of death before age 70 in 91 out of 172 countries. There were approximately 18.1 million new cancer cases and 9.6 million cancer deaths in 2018 worldwide (Bray et al., 2018). Furthermore, there were approximately 145,600 new cases of colorectal cancer (CRC) and 51,020 CRC-related deaths in the United States in 2019 (Siegel et al., 2019). The observed reductions in CRC incidence prior to 2000 are attributed to increased screening and the smaller but demonstrable impacts of risk-factor reduction and improved treatment (Edwards et al., 2010). Therefore, the early detection of CRC is important for implementing individualized treatment to achieve a favorable prognosis.

Aldehyde dehydrogenase isoform 1 (ALDH1), which is a cytosolic enzyme ubiquitously distributed in tissues and cells including the brain and red blood cells, may be required for the formation of retinoic acid, a potent modulator of gene expression and tissue differentiation (Yoshida et al., 1998). Furthermore, ALDH1 serves as a functional marker of cancer stem cells and progenitor cells (Douville et al., 2009). ALDH1 is highly expressed in CRC, hepatocellular carcinoma, esophageal cancer, cervical cancer, and lung cancer (Ajani et al., 2014; Huo et al., 2015; Li et al., 2016; Yang et al., 2017; Liu et al., 2020). The ALDH1 family comprises the isoforms ALDH1A1, ALDH1A2, ALDH1A3, ALDH1B1, ALDH1L1, and ALDH1L2 (Liang et al., 2019). ALDH1B1 resides in the mitochondria and metabolizes diverse aldehyde substrates such as acetaldehyde and products of lipid peroxidation (Stagos et al., 2010).

Analyses of *ALDH1B1* mRNA and protein levels, as well as ALDH1B1 cDNA sequences of CRC cell lines,

indicate that *ALDH1B1* is not genetically altered in tumors. Analysis of tissue microarrays shows that ALDH1B1 is differentially expressed in CRC tissues versus normal tissues (Matsumoto et al., 2017). For example, ALDH1B1 is expressed at 5.6-fold higher levels in cancer tissues than ALDH1A1 (Chen et al., 2011). Moreover, analyses of a three-dimensional spheroid growth model and a nude mouse xenograft tumor model show that ALDH1B1 is required for colon tumorigenesis through its modulation of the Wnt/β-Catenin, Notch, and PI3K/Akt signaling pathways (Singh et al., 2015).

We therefore hypothesized that ALDH1B1 expression is associated with colorectal tumors. To test this hypothesis, we used a commercial colorectal tissue microarray as well as clinical CRC and control samples collected at our institution to evaluate the role of ALDH1B1 expression in colorectal adenoma and CRC. Moreover, we used the online data-mining platform, Oncomine, to analyze the expression of *ALDH1B1* mRNA. The associations of ALDH1B1 with the stage and prognosis of CRC were evaluated using the online tools, GEPIA and UALCAN.

Materials and methods

Patients and tissue samples

A commercial tissue microarray was purchased from Shanghai Outdo Biotech Co., Ltd. (HLinDis060PT01; Shanghai, China). Clinical diagnoses and the TNM (tumor, nodes, and metastasis staging system) scores were acquired from the patients' clinical data provided by the company. The tissue microarray contained 37 colon tissues (5 normal, 24 adenomas, and 4 tumor tissues with 4 matched cancer-adjacent colon tissues) and 23 rectal tissues (2 normal, 11 adenomas, and 5 tumor tissues with 5 matched cancer-adjacent rectal tissues). The microarray sample information is described in detail in Table 1. Colonic tissue samples included those from 23 males and 14 females with a median age of 58. Rectal tissue samples included those from 10 males and 14 females with a median age of 50. The detailed patient information is presented in Table 2. We also obtained 10 CRC tissue samples (6 males and 4 females with a median age of 65 years) with 10 matched pairs of paracancerous tissue samples and 3 colorectal adenomas (3 males with a median age of 55 years) from the Department of General Surgery, Beijing Friendship Hospital, Capital Medical University. The clinical sample information is shown in Table 3. One adenoma patient was diagnosed with tubular adenoma, villous adenoma, and villous-tubular adenoma. The 10 CRC tissues were confirmed by pathological examination for inclusion in the study (Table 4). The Union for International Cancer Control (UICC) TNM staging system was applied according to the eighth edition of the Cancer Staging Manual. This study was approved by the Ethics Committee (approval no. 2018-P2-045-01) of the Beijing Friendship Hospital, Capital Medical University. All participants provided written informed consent.

Immunohistochemical analysis of the commercial colorectal tissue microarray and the institutional samples

The protein expression of ALDH1B1 in the tissue samples was evaluated using immunohistochemistry (IHC), which was performed based on the standard streptavidin-peroxidase method. Briefly, histologic slides of the tissue samples were deparaffinized through the application of xylene and an alcohol gradient. The antigen was retrieved with citric acid antigen repair buffer, pH 6.0 (ZSGB-BIO, Beijing, China). The endogenous peroxidase activity was blocked by incubating the slides in a 3% H_2O_2 solution (Kit I, ZSGB-BIO) prepared in methanol at room temperature for 10 min. The slides were washed with phosphate-buffered saline (PBS, Ph7.4) and then blocked using blocking buffer (10% fetal bovine serum in phosphate-buffered saline). The slides were subsequently incubated with a rabbit anti-human polyclonal antibody against ALDH1B1 (ab246928, dilution of 1:1,000; Abcam, Cambridge, UK) at 4°C overnight. Next, the slides were washed and incubated with the polyclonal anti-rabbit IgG secondary antibody (Kit II, ZSGB-BIO). Immuno-complexes were detected using 3,3'-diaminobenzidine (DAB; ZSGB-BIO). Staining was monitored under a microscope and terminated when sufficient staining was achieved. The slides were then dehydrated and stored. Images of the tissues were acquired using a visible-light microscope (200× magnification). The intensity of specific staining was measured using Image-Pro Plus 6.0 software (Media Cybernetics, Silver Spring, MD, USA). All images were acquired using the same microscope and camera set. The intensities of the positive staining in the cytoplasm and membranes were determined using the mean integrated optical density (mean IOD) as follows: mean IOD=IOD/area of the tumor section.

Oncomine analysis of ALDH1B1 expression

Oncomine is a cancer microarray database and web-based data-mining platform (http://www.oncomine.org) that comprises 715 datasets and 86,733 samples to facilitate discovery using genome-wide expression analyses (Rhodes et al., 2004). Here, we used Oncomine to analyze the levels of *ALDH1B1* mRNA expression in different types of cancers and colorectal tumors. *ALDH1B1* levels in clinical cancer specimens were compared with those of normal controls. The Student's t-test was used to generate p values. The cut-off value for p and the fold-change were set at 0.05 and 2, respectively.

Analysis of ALDH1B1 gene expression and its association with CRC stage using GEPIA

GEPIA (http://gepia.cancer-pku.cn/index.html) is a

commonly used interactive tool (Tang et al., 2017). The GEPIA dataset, which comprises samples from 9,736 tumors and 8,587 normal tissues from The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) databases, was used to determine the expression levels of *ALDH1B1* and any associations

Table 1. Clinicopathological characteristics of 60 colorectal tissues in microarray.

Numbers	Age/gender	Pathologic diagnosis	UICC stage	Location
A1	42/M	Normal colonic tissue		Colon
A2	56/M	Normal colonic tissue		Colon
A3	38/M	Normal colonic tissue		Colon
A4	66/M	Normal colonic tissue		Colon
A5	45/M	Normal colonic tissue		Colon
A6	58/M	Tubular adenoma		Colon
A7	42/F	Tubular adenoma		Colon
A8	62/M	Tubular adenoma		Colon
A9	55/M	Villous adenoma		Colon
A10	73/M	Villus-tubular adenoma		Colon
B1	38/M	Villus-tubular adenoma		Colon
B2	84/M	Villus-tubular adenoma		Colon
B3	49/F	Villus-tubular adenoma		Colon
B4	44/M	Tubular adenoma, intraepithelial neoplasia		Colon
B5	69/M	Tubular adenoma, intraepithelial neoplasia		Colon
B6	40/F	Tubular adenoma, intraepithelial neoplasia		Colon
B7	47/F	Villous adenoma, intraepithelial neoplasia		Colon
B8	54/M	Villus-tubular adenoma, intraepithelial neoplasia		Colon
B9	63/M	Villus-tubular adenoma, intraepithelial neoplasia		Colon
B10	47/F	Villus-tubular adenoma, intraepithelial neoplasia		Colon
C1	58/M	Villus-tubular adenoma, intraepithelial neoplasia		Colon
C2	68/F	Villus-tubular adenoma, intraepithelial neoplasia		Colon
C3	44/M	Villus-tubular adenoma, intraepithelial neoplasia		Colon
C4	58/F	Villus-tubular adenoma, intraepithelial neoplasia		Colon
C5	78/F	Villus-tubular adenoma, intraepithelial neoplasia		Colon
C6	60/M	Villus-tubular adenoma, intraepithelial neoplasia		Colon
C7	69/M	Villus-tubular adenoma, intraepithelial neoplasia		Colon
C8	69/F	Villus-tubular adenoma, intraepithelial neoplasia		Colon
C9	72/F	intraepithelial neoplasia		Colon
C10	71/M	Adenocarcinoma	pT3N0M0	Colon
D1	71/M	Cancer adjacent colon tissue		Colon
D2	87/M	Adenocarcinoma	pT3N0M0	Colon
D3	87/M	Cancer adjacent colon tissue		Colon
D4	38/F	Adenocarcinoma	pT4aN0M0	Colon
D5	38/F	Cancer adjacent colon tissue		Colon
D6	42/F	Adenocarcinoma	pT4bN2aM0	Colon
D7	42/F	Cancer adjacent colon tissue		Colon
D8	59/M	Normal rectal tissue		Rectum
D9	47/M	Normal rectal tissue		Rectum
D10	50/F	Villus-tubular adenoma		Rectum
E1	39/F	Villus-tubular adenoma		Rectum
E2	59/F	Villus adenoma, intraepithelial neoplasia		Rectum
E3	68/M	Villus adenoma, intraepithelial neoplasia		Rectum
E4	78/M	Villus adenoma, intraepithelial neoplasia		Rectum
E5	76/F	Villus adenoma, intraepithelial neoplasia		Rectum
E6	73/M	Villus-tubular adenoma, intraepithelial neoplasia		Rectum
E7	60/M	Villus-tubular adenoma, intraepithelial neoplasia		Rectum
E8	73/F	Villus-tubular adenoma, intraepithelial neoplasia		Rectum
E9	74/M	Villus-tubular adenoma, intraepithelial neoplasia		Rectum
E10	72/M	Villus-tubular adenoma, intraepithelial neoplasia		Rectum
F1	84/F	Adenocarcinoma	pT3N0M0	Rectum
F2	84/F	Cancer adjacent rectum tissue		Rectum
F3	32/F	Adenocarcinoma	pT4aN0M0	Rectum
F4	32/F	Cancer adjacent rectum tissue		Rectum
F5	54/F	Adenocarcinoma	pT2N0M0	Rectum
F6	54/F	Cancer adjacent rectum tissue		Rectum
F7	40/F	Adenocarcinoma	pT3N1bM0	Rectum
F8	40/F	Cancer adjacent rectum tissue		Rectum
F9	50/M	Adenocarcinoma	pT4bN2aM1	Rectum
F10	50/M	Cancer adjacent rectum tissue		Rectum

with CRC stage. The log-rank test was performed and the resulting HR and p values or Cox p values from the log-rank test were plotted.

Analysis of prognosis associated with ALDH1B1 expression using UALCAN and GEPIA

UALCAN (http://ualcan.path.uab.edu/) is a comprehensive web resource for analyzing cancer data (Chandrashekar et al., 2017). UALCAN generates graphs depicting gene expression and survival curves, evaluates the methylation of promoter sequences, and performs pan-cancer gene expression analysis. Here, we used UALCAN to mine survival data for patients with CRC samples that were associated with *ALDH1B1* expression. GEPIA was then used to validate the correlation between *ALDH1B1* expression and either overall survival (OS) or disease-free survival (DFS).

Results

ALDH1B1 is highly expressed in colorectal adenomas and CRCs

We used a commercial tissue microarray to measure the protein expression of ALDH1B1 at 60 sites, including colorectal tumors and normal tissue samples. The microarray sample information is described in detail in Table 1, and the detailed clinicopathological characteristics and mean IODs of all 60 sites are shown in Table 5. The tissue matrix diagram and IHC results are presented in Fig. 1. The corresponding IHC data from the colonic and rectal tissues are presented in Figures 2 and 3, respectively. Positive staining was observed in both the cytoplasmic and membranous areas. The expression of ALDH1B1 was significantly higher in the colorectal adenomas and colorectal adenocarcinoma (IOD/area values = 0.117±0.070 and 0.168±0.0168, respectively) compared with normal and cancer-adjacent tissues (IOD/area values = 0.051±0.028 and 0.068±0.053, respectively; $p<0.01$, $p=0.007$). In the colonic tissues, higher ALDH1B1 expression was detected in the adenocarcinoma tissues (IOD/area = 0.218±0.088) compared with the adenoma tissues (IOD/area = 0.129±0.072), normal colonic tissues (IOD/area = 0.042±0.029), and cancer-adjacent colon tissues (IOD/area = 0.107±0.059). ALDH1B1 expression in colonic adenoma (IOD/area = 0.129±0.072) was more than in normal colonic tissues (IOD/area = 0.042±0.0029, $p=0.004$). In the rectal tissues, ALDH1B1 expression was higher in the adenocarcinoma tissues (IOD/area = 0.129±0.059) compared with normal rectal tissues (IOD/area = 0.074±0.001) and cancer-adjacent rectal tissues (IOD/area = 0.037±0.015). It was statistic difference when comparing rectal adenocarcinoma and cancer-adjacent rectal tissues ($p=0.01$). Furthermore, ALDH1B1 expression in tubular (IOD/area = 0.191±0.038) and villous-tubular adenomas (IOD/area =

Table 2. Clinical characteristics of tissue microarray.

	Dot numbers	Age mean	Median (range)	Gender (male/female)
Colonic tissue	37	57	58 (38-87)	23/14
Normal colonic tissue	5	49	45 (38-66)	5/0
Adenoma	24	58	58 (42-84)	14/10
Adenocarcinoma/ Cancer adjacent colon tissue	4	60	57 (42-87)	2/2
Rectal tissue	23	52	50 (32-84)	10/13
Normal rectal tissue	2	53	53 (47-59)	2/0
Adenoma	11	49	49 (39-78)	6/5
Adenocarcinoma/ Cancer adjacent rectum tissue	5	52	50 (32-84)	1/4

Table 3. Clinicopathological characteristics of colorectal tissues collected in hospital.

Numbers	Age/gender	Pathologic diagnosis	UICC stage	Location
1	63/M	Tubular adenoma, Villous adenoma, Villus-tubular adenoma		Colon
2	55/M	Villous adenoma		Colon
3	43/M	Tubular adenoma		Colon
4	68/F	Adenocarcinoma	ypT3N0M0	Rectum
5	60/M	Adenocarcinoma	pT2N0M0	Colon
6	78/M	Adenocarcinoma	pT3N0M0	Colon
7	72/F	Adenocarcinoma	pT3N2bM0	Colon
8	62/M	Adenocarcinoma	pT3N1cM0	Rectum
9	70/M	Adenocarcinoma	pT3N1aM0	Colon
10	72/F	Myxoma	pT3N0M0	Colon
11	54/F	Adenocarcinoma	pT2N0M0	Rectum
12	58/F	Adenocarcinoma	pT4aM0N0	Rectum
13	37/M	Adenocarcinoma	pT3N0M0	Colon

0.112±0.078) with intraepithelial neoplasia compared with adenomas without intraepithelial neoplasia (IOD/area = 0.109±0.103, IOD/area = 0.144±0.104) did not have evident differences. The expression of ALDH1B1 was uniformly low in villus adenoma with or without intraepithelial neoplasia (IOD/area = 0.048, IOD/area = 0.065±0.035, respectively) compared with other adenomas and adenocarcinomas.

To further investigate the expression of ALDH1B1 in adenomas and CRC tissues, we analyzed adenomas, CRC tissues, and cancer-adjacent tissues from our institution's collection. One of the adenoma patients included was diagnosed with tubular adenoma, villous adenoma, and villous-tubular adenoma. All tissues from this patient were stained at the same time and analyzed according to standard methods for the pathological diagnosis of adenoma. The detailed clinicopathological characteristics and mean IODs of the colorectal tissues are presented in Table 6, and the IHC results are shown in Figure 4. The IHC data were generally consistent with those of the commercial tissue microarray and revealed that ALDH1B1 was highly expressed in the adenoma (IOD/area = 0.103±0.054) and CRC (IOD/area = 0.116±0.059) tissues compared with the cancer-adjacent tissues (IOD/area = 0.066±0.024, $p<0.05$). The levels of ALDH1B1 in the tubular adenomas (IOD/area = 0.141±0.003) and villous-tubular adenomas (IOD/area = 0.105±0.052) were higher than in the villous adenomas (IOD/area = 0.025).

The combined CRC tissue microarray and institutional samples represented CRC stages I (n=3), II (n=9), III (n=5), and IV (n=1). The levels of ALDH1B1 expression in the stage I and II samples were not significantly different (IOD/area=0.098±0.064 vs. 0.167±0.082, $p>0.05$). There was also no significant difference between stage II and III samples (IOD/area = 0.167±0.082 vs. 0.161±0.052, $p>0.05$). The mean IOD of stage IV was 0.088. These findings indicate that the expression levels of ALDH1B1 were not significantly associated with CRC stage.

Transcriptional levels of ALDH1B1 in patients with CRC

We employed the Oncomine platform to compare the transcriptional levels of *ALDH1B1* in normal samples with those in tumor samples. There were 432 unique analyses of all tumors, including 14 and 10 analyses showing upregulated or downregulated expression of *ALDH1B1*, respectively (Fig. 5a). The expression of *ALDH1B1* mRNA was significantly upregulated in seven out of the 14 analyses.

We next determined the levels of *ALDH1B1* mRNA in colorectal tumor tissues. We employed 11 datasets for the analysis of *ALDH1B1* expression (Table 7). There were no significant differences in the levels of ALDH1B1 mRNA expression between colorectal adenocarcinomas from the Notterman colon and Ki colon datasets and normal tissues, between rectal mucinous adeno-carcinomas from the TCGA colorectal

Table 4. The characteristics of colorectal cancer and colorectal adenoma collected in in hospital.

	Numbers	Age mean	Median (range)	Gender (male/female)
Colorectal cancer	10	63	65 (37-78)	6/4
UICC stage I	2	57	57 (54-60)	1/1
UICC stage II	5	63	68 (37-78)	2/3
UICC stage III	3	68	70 (62-72)	3/0
UICC stage IV	0	-	-	-
Colorectal adenoma	3	54	55 (43-63)	3/0

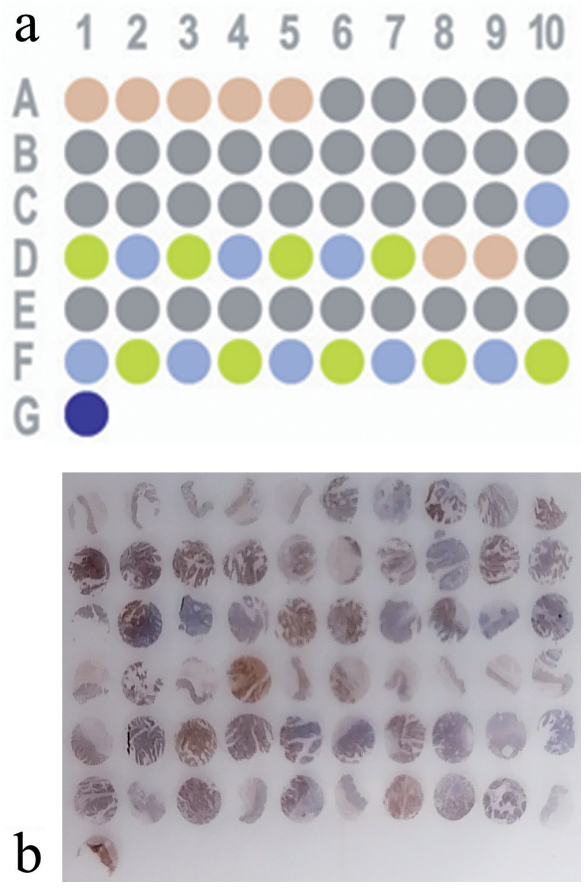

Fig. 1. Tissue matrix diagram of the tissue microarray **(a)** and overall immunohistochemistry results **(b)**. A1-D7, colonic tissues; A1-A5, normal colonic tissues; A6-A8, tubular adenomas; A9, villous adenomas; A10-B3, villous-tubular adenomas; B4-B6, tubular adenomas with intraepithelial neoplasia; B7, villous adenomas with intraepithelial neoplasia; B8-C8, villous-tubular adenomas with intraepithelial neoplasia; C9, intraepithelial neoplasia; C10-D7, adenocarcinomas and cancer-adjacent colon tissues; D8-D9, normal rectal tissues; D10-E1, villous-tubular adenomas; E2-E5, villous adenomas with intraepithelial neoplasia; E6-E10, villous-tubular adenomas with intraepithelial neoplasia; F1-F10, adenocarcinomas and cancer-adjacent rectal tissue.

Table 5. Clinicopathological characteristics and mean integrated optic density (IOD) of 60 colorectal tissues.

	No. of samples	Mean IOD
Normal colorectal tissue	7	0.051±0.028
Colorectal adenoma	35	0.117±0.070
Colorectal adenocarcinoma	9	0.168±0.083
Cancer adjacent tissue	9	0.068±0.053
Colonic tissue	37	0.125±0.079
Normal colonic tissue	5	0.042±0.029
Adenoma	24	0.129±0.072[a]
Tubular adenoma	3	0.109±0.103
Villous adenoma	1	0.048
Villus-tubular adenoma	4	0.165±0.031
Tubular adenoma, intraepithelial neoplasia	3	0.191±0.038
Villous adenoma, intraepithelial neoplasia	1	0.064
Villus-tubular adenoma, intraepithelial neoplasia	11	0.116±0.078
Intraepithelial neoplasia	1	0.148
Adenocarcinoma	4	0.218±0.088[b]
Cancer adjacent colon tissue	4	0.107±0.059[c]
Rectal tissue	23	0.085±0.058
Normal rectal tissue	2	0.074±0.001
Adenoma	11	0.089±0.062[d]
Villus-tubular adenoma	2	0.102±0.001
Villus adenoma, intraepithelial neoplasia	4	0.065±0.041
Villus-tubular adenoma, intraepithelial neoplasia	5	0.104±0.087
Adenocarcinoma	5	0.129±0.059[e]
Cancer adjacent rectum tissue	5	0.037±0.015f

Data presented as mean ± SD. [a]: colonic adenoma versus normal colonic tissue (p=0.015); [b]: colonic adenocarcinoma versus normal colonic tissue (p=0.004); [c]: colonic adenocarcinoma versus cancer adjacent colon tissue (p=0.080); [d]: rectal adenoma versus normal rectal tissue (p=0.743); [e]: rectal adenocarcinoma versus normal rectal tissue (p=0.108); [f]: rectal adenocarcinoma versus cancer adjacent colon tissue (p=0.01).

and Kaiser colon datasets and normal tissues, or between that of normal tissue and cecum adenocarcinomas, rectosigmoid adenocarcinomas, and rectal adenocarcinomas in several datasets. When we compared all of the relevant datasets, *ALDH1B1* mRNA was significantly overexpressed (p=0.024) in colorectal tumors (Fig. 5b), and there were no findings of significantly lower differential levels (p=0.981) (Fig. 5c).

The relationship between ALDH1B1 mRNA expression and CRC stage

When using GEPIA to analyze *ALDH1B1* mRNA

Table 6. Clinicopathological characteristics and mean integrated optic density (IOD) of colorectal tissues collected at our hospital.

	No. of samples	Mean IOD
Adenoma	5[a]	0.103±0.054
Tubular adenoma	2	0.141±0.003
Villous adenoma	1	0.025
Villus-tubular adenoma	2	0.105±0.052
Colorectal adenocarcinoma	9	0.116±0.059
Cancer adjacent tissue	9	0.066±0.024
Colonic adenocarcinoma	5	0.116±0.038[b]
Cancer adjacent colon tissue	5	0.061±0.032
Rectal adenocarcinoma	4	0.116±0.043[c]
Cancer adjacent rectum tissue	4	0.062±0.014
Colonic myxoma	1	0.124
Colonic myxoma adjacent colon tissue	1	0.091

[a]: One adenoma patient was diagnosed with tubular adenoma, villous adenoma, and villous-tubular adenoma. All three tissues from this patient were stained at the same time and analyzed according to standard methods for the pathological diagnosis of adenoma. [b]: Colonic adenocarcinoma versus Cancer adjacent colon tissue (p=0.042); [c]: Colonic adenocarcinoma versus Cancer adjacent colon tissue (p=0.045).

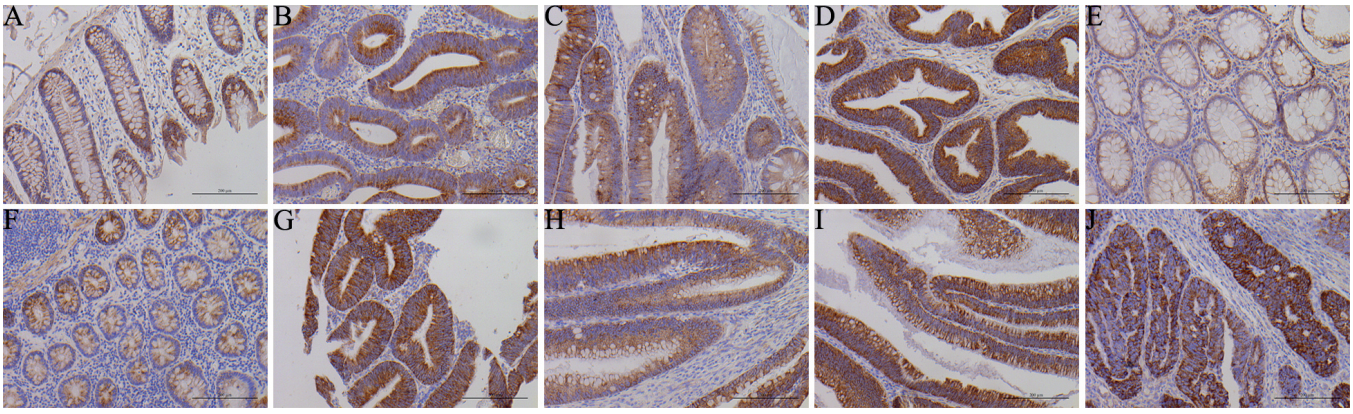

Fig. 2. Immunohistochemical analysis of a commercial tissue microarray of colonic tissues. **A.** Colonic tissue. **B.** Tubular adenoma. **C.** Villous adenoma. **D.** Villous-tubular adenoma. **E.** Cancer-adjacent colon tissue. **F.** Intraepithelial neoplasia. **G.** Tubular adenoma with intraepithelial neoplasia. **H.** Villous adenoma with intraepithelial neoplasia. **I.** Villous-tubular adenoma with intraepithelial neoplasia. **J.** Colonic adenocarcinoma. Scale bars: 200 μm.

Table 7. Oncomine analysis of ALDH1B1 mRNA expression in colorectal tumors.

Cohort	Sample (n)	Fold change	P value
Sabates-Bellver	Colon Adenoma (25) vs Normal (32)	2.986	1.54E-15
Skrzypczak Colorectal	Colorectal Adenomacarcinoma (45) vs Normal (24)	2.377	1.43E-11
Skrzypczak Colorectal 2	Colon Adenoma (5) vs Normal (10)	4.565	1.55E-6
	Colon Carcinoma (5) vs Normal (10)	3.632	1.98E-7
	Colon Adenoma Epithelia (5) vs Normal (10)	2.863	5.33E-5
Gaedcke Colorectal	Rectal Adenomacarcinoma (65) vs Normal (65)	2.105	1.82E-22
Hong Colorectal	Colorectal carcinoma (70) vs Normal (12)	2.079	1.77E-8
			1.54E-15
Gaspar Colon	Colon Adenoma Epithelia (56) vs Normal (22)	1.565	1.67E-5
Notterman Colon	Colorectal Adenomacarcinoma (18) vs Normal (18)	-1.140	0.811
Ki Colon	Colorectal Adenomacarcinoma (50) vs Normal (28)	1.140	0.098
TCGA Colorectal	Cecum Adenomacarcinoma (22) vs Normal (22)	1.574	7.17E-5
	Rectosigmoid Adenomacarcinoma (22) vs Normal (22)	2.123	0.024
	Rectal Adenomacarcinoma (60) vs Normal (22)	1.466	9.81E-5
	Colon Mucinous Adenomacarcinoma (22) vs Normal (22)	1.443	0.004
	Colon Adenomacarcinoma (101) vs Normal (22)	1.343	3.98E-4
	Rectal Mucinous Adenomacarcinoma (6) vs Normal (22)	1.567	0.085
Graudens Colon	Colon Adenomacarcinoma (18) vs Normal (12)	-1.125	0.955
Kaiser Colon	Colon Mucinous Adenomacarcinoma (41) vs Normal (5)	1.264	0.014
	Colon Mucinous Adenomacarcinoma (13) vs Normal (5)	1.239	0.046
	Cecum Adenomacarcinoma (17) vs Normal (5)	1.096	0.252
	Rectosigmoid Adenomacarcinoma (10) vs Normal (5)	1.040	0.381
	Rectal Adenomacarcinoma (8) vs Normal (5)	1.026	0.441
	Rectal Mucinous Adenomacarcinoma (4) vs Normal (22)	-1.022	0.526

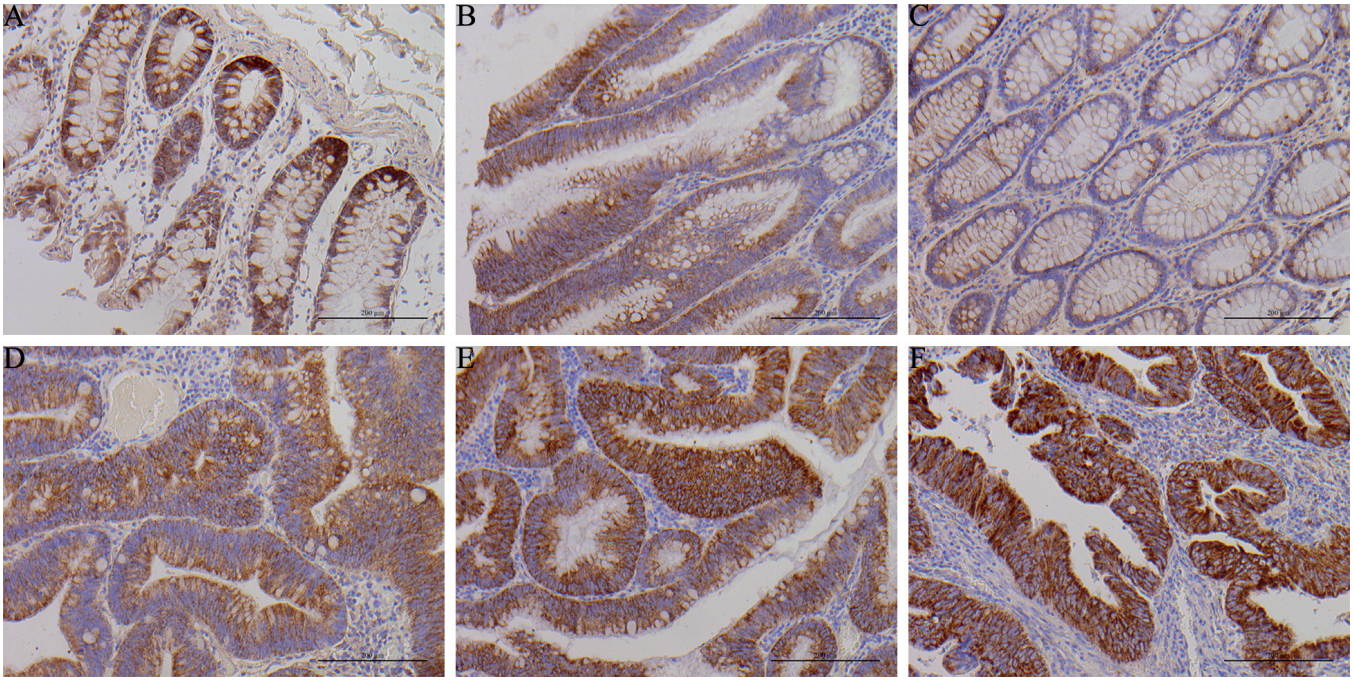

Fig. 3. Immunohistochemical analysis of a commercial tissue microarray of rectal tissues. **A.** Normal rectal tissue. **B.** Villous-tubular adenoma. **C.** Cancer-adjacent rectal tissue. **D.** Villous adenoma. **E.** Villous-tubular adenoma with intraepithelial neoplasia. **F.** Rectal adenocarcinoma. Scale bars: 200 μm.

expression, 275 cases of colon cancer, 92 cases of rectal cancer, and 667 samples of normal colorectal tissues were included. This analysis revealed that *ALDH1B1* mRNA expression was higher in these cancers than in normal colorectal tissues (Fig. 5a). However, *ALDH1B1* expression in CRC tissues was not significantly associated with CRC stage ($p>0.05$) (Fig. 6b). These findings indicate that the mRNA expression levels of *ALDH1B1* are independent of CRC stage.

The relationship between ALDH1B1 mRNA and CRC prognosis

The relationship between the mRNA expression levels of *ALDH1B1* and the prognosis of patients with CRC was analyzed using UALCAN and GEPIA. *ALDH1B1* expression was not significantly associated with the survival of patients with colon or rectal cancers ($p=0.21$ and $p=0.64$, respectively) (Fig 7a, b). When using GEPIA to evaluate the relationship between *ALDH1B1* mRNA expression and CRC prognosis, we did not find any significant differences in the OS and DFS between the normal population and patients with CRC ($p=0.29$ and $p=0.33$, respectively) (Fig. 7c,d).

Discussion

CRC is one of the most commonly diagnosed cancers worldwide and the second leading cause of adult deaths in the United States (Kastenberg et al., 2018). CRC may present with an identifiable premalignant lesion that progresses with a characteristic timeline, which facilitates effective treatment (Curtius et al., 2017).

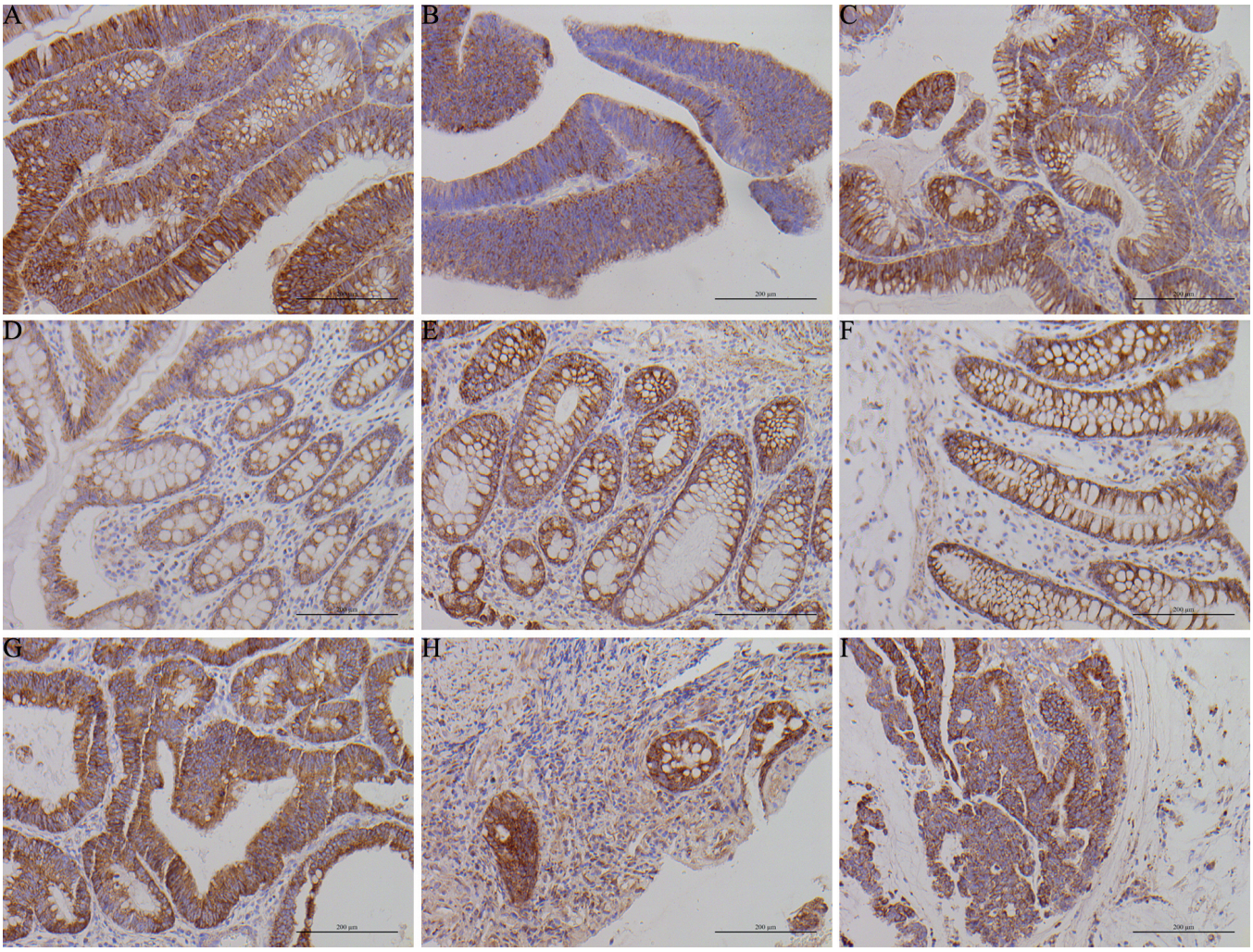

Fig. 4. Immunohistochemical analysis of institutional colorectal tissues. **A.** Tubular adenoma. **B.** Villous adenoma. **C.** Villous-tubular adenoma. **D.** Cancer-adjacent colon tissue. **E.** Cancer-adjacent rectum tissue. **F.** Cancer-adjacent colon tissue to colonic myxoma. **G.** Colonic adenocarcinoma. **H.** Rectal adenocarcinoma. **I.** Colonic myxoma. Scale bars: 200 μm.

ALDH1, which serves as a marker of stem cells of diverse cancers, modulates cell proliferation, stem cell differentiation, and resistance to chemotherapeutics. ALDH1B1 contributes toward ALDH1 activity (Yang et al., 2017). ALDH1B1 may promote the differentiation of stem cells (Jackson et al., 2015). For example, an analysis of tissue arrays found that ALDH1B1 expression is dramatically upregulated in human colonic adenocarcinomas (Chen et al., 2011). Other tissue microarray findings show that ALDH1B1 levels distinguish CRC tissues from normal tissues (Matsumoto et al., 2017). In the present study, although the number of tissue samples was limited, we observed a significant association between ALDH1B1 protein expression and colorectal tissues and adenomas.

Here, we also aimed to determine whether the expression of ALDH1B1 in colorectal tumors was significantly associated with cancer stage and prognosis. We found that ALDH1B1 was expressed at significantly higher levels in colorectal adenomas and CRC tissues

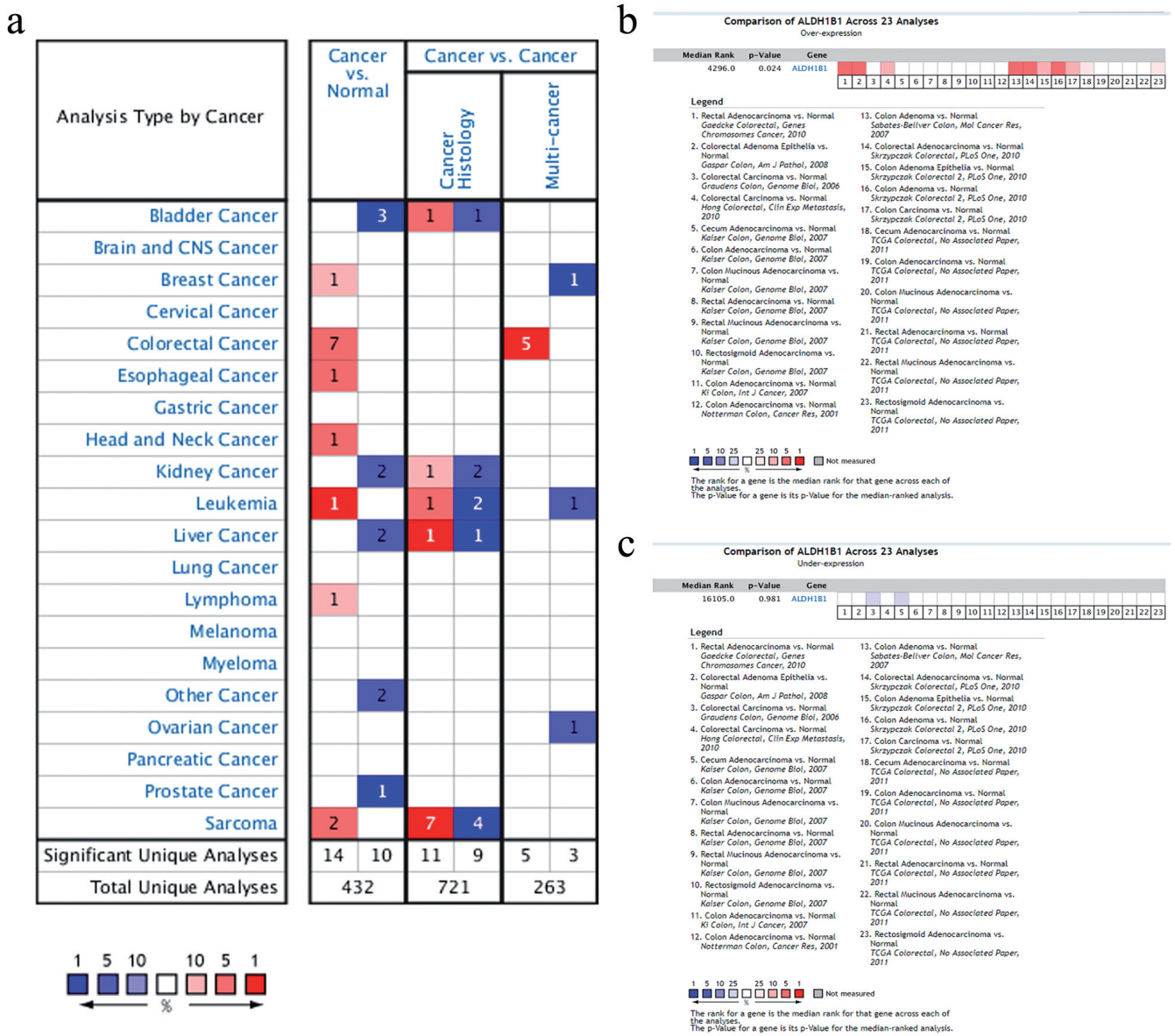

Fig. 5. Expression of *ALDH1B1* mRNA in colorectal cancer. **a.** Overview of the *ALDH1B1* mRNA expression in tumors as analyzed by Oncomine. **b.** *ALDH1B1* mRNA levels in Oncomine datasets where its expression was significantly increased in colorectal tumor tissues compared with normal colorectal tissues (p=0.024). **c.** *ALDH1B1* mRNA levels in Oncomine datasets where its expression was lower in colorectal tumor tissues than in normal colorectal tissues. However, this difference was not statistically significant (p=0.981).

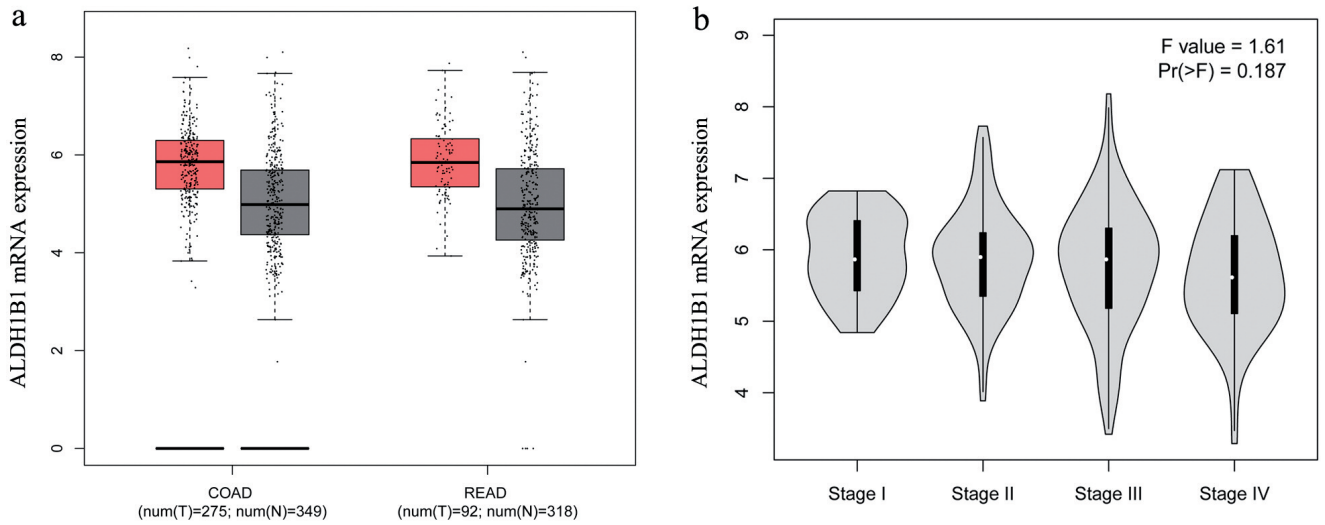

Fig. 6. Expression of *ALDH1B1* mRNA related to the stages of colorectal cancer. **a.** The expression of *ALDH1B1* in colon adenocarcinoma (COAD) and rectum adenocarcinoma (READ) patients. **b.** Correlation between *ALDH1B1* expression and the tumor stages of patients with colorectal cancer.

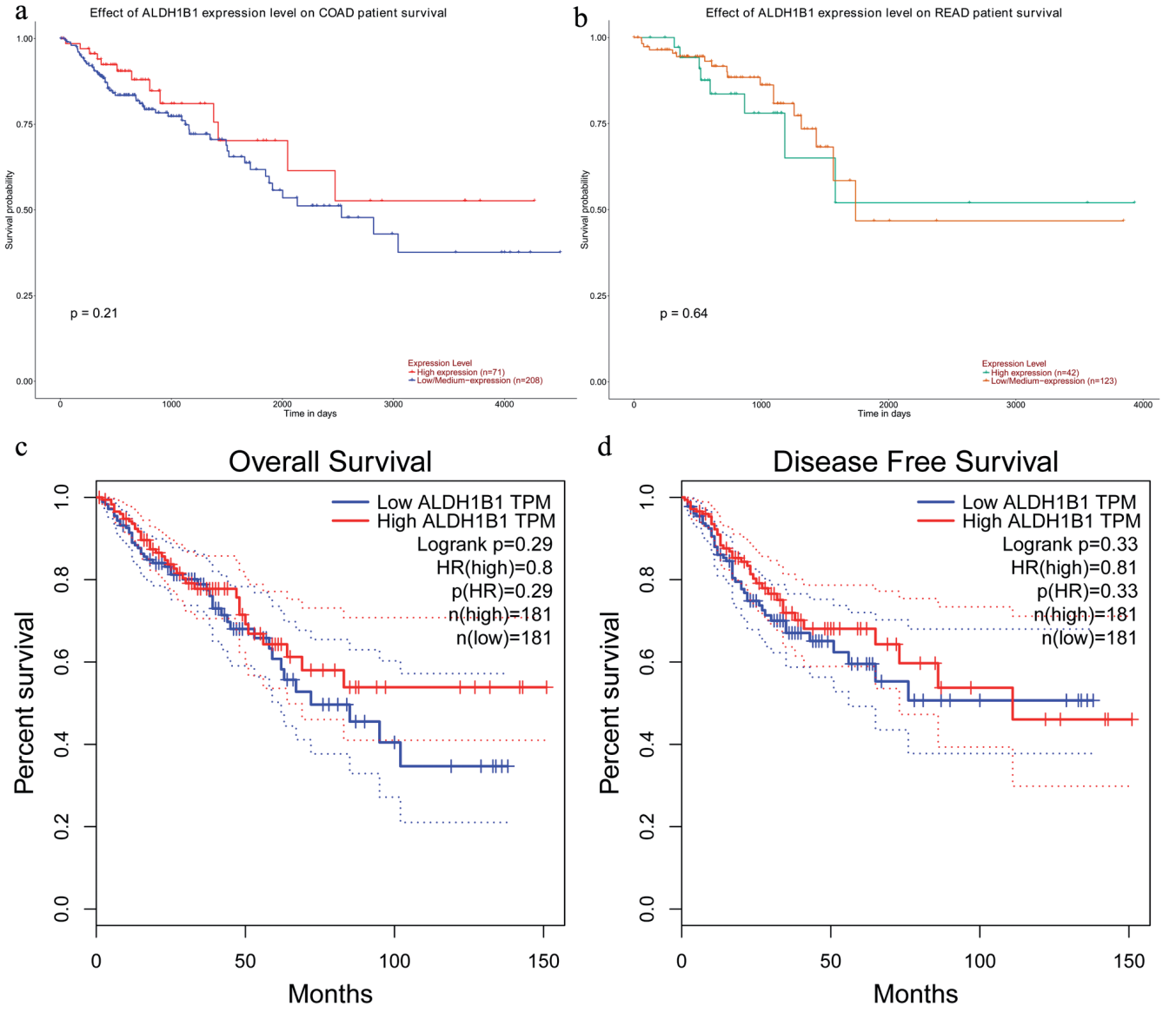

Fig. 7. Expression of *ALDH1B1* mRNA related to the prognosis of patients with colorectal cancer. The association between the expression of *ALDH1B1* and the survival of colon adenocarcinoma (COAD) **(a)** and rectum adenocarcinoma (READ) **(b)** patients were analyzed in UALCAN. **c-d.** Correlation between *ALDH1B1* expression and the prognosis of patients with colorectal cancer as determined with GEPIA.

compared with normal colorectal tissues and cancer-adjacent tissues. Furthermore, ALDH1B1 was expressed at higher levels in tubular and villous-tubular adenomas than in villous adenomas.

Studies show that ALDH1 serves as a specific marker for identifying, isolating, and tracking human colonic stem cells during the development of CRC. Moreover, mathematical modeling of crypt stem cell dynamics shows that progressive overpopulation of colonic stem cells occurs during colon tumorigenesis and drives the development of CRC (Huang et al., 2009). We reasoned, therefore, that ALDH1B1 contributes toward colon tumorigenesis. To test this hypothesis, we used Oncomine to determine the levels of *ALDH1B1* mRNA expression in colorectal tumor tissues. We found that *ALDH1B1* was significantly upregulated in colorectal tumor tissues compared with normal tissues, and GEPIA analysis yielded findings consistent with those of the Oncomine analysis. When we analyzed a limited number of CRC tissues, we found that the protein expression of *ALDH1B1* was not significantly associated with CRC stage. Furthermore, GEPIA analysis revealed that the levels of *ALDH1B1* mRNA expression in CRC were also independent of disease stage.

ALDH1 indicates stemness and serves as a biomarker for colon cancer; however, its expression levels do not increase during the progression from the normal to malignant phenotypes of colon epithelial cells (Fitzgerald et al., 2014). A pilot study has suggested an association between ALDH1B1 expression and the differentiation of CRC cells, although an association between ALDH1B1 expression and disease stage was not observed (Langan et al., 2012). Another study did not find any association between ALDH1B1 levels and cancer stage (TNM II/III vs. TNM IV) (Matsumoto et al., 2017). Furthermore, our present study of CRC using UALCAN and GEPIA analysis did not find a significant association between the levels of *ALDH1B1* expression and prognosis.

Our study has certain limitations. First, the relationship between ALDH1B1 expression in CRC tissues and disease stage was not statistically significant. Although our sample sizes were small, GEPIA analysis also supported this finding. Second, we did not follow up on the survival of patients who supplied CRC tissues to confirm or disprove a correlation between ALDH1B1 expression and CRC prognosis. Furthermore, we did not investigate the mechanism of ALDH1B1 overexpression in colorectal tumors. Further studies are, therefore, required.

In summary, in this study, we systemically analyzed the expression and prognostic value of ALDH1B1 expression in colorectal tumors as a potential marker for colorectal tumors, particularly for adenomas. Our integrated bioinformatic analyses revealed that although the protein and mRNA expression of ALDH1B1 was higher in colorectal tumors, it was independent of CRC stage and prognosis.

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Author contributions. Jian Huang and Hejing Wang contributed to the study design. Siyu Jia and Saiping Qi assisted with sample collection. Hejing Wang, Yanmeng Li, and Donghu Zhou performed the experiments. Jian Huang and Hejing Wang contributed significantly toward drafting the manuscript, literature review, and critical revision. All authors read and approved the final manuscript.

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