Pathol. Oncol. Res. (2016) 22:121–127
DOI 10.1007/s12253-015-9984-x

ORIGINAL ARTICLE

Aldehyde Dehydragenase 1 and Nodal as Significant Prognostic Markers in Colorectal Cancer

Hui Li[1] · Yang Jiang[2] · Fenghua Pei[1] · Lu Li[1] · Bingzhu Yan[3] · Xinyu Geng[1] · Bingrong Liu[1]

Received: 18 June 2015 / Accepted: 3 September 2015 / Published online: 10 September 2015

Abstract This study aimed to analyze prognostic significance of aldehyde dehydragenase 1 (ALDH1) and Nodal expression in patients with colorectal cancer. ALDH1 and Nodal expressions were observed based on the immunohistochemistry staining from 108 colorectal cancer patients. Scores were given to the staining intensity and percentage of positive cells, and sum of two scores for each case was used to define the groups of ALDH1 and Nodal. We also investigated the protein and mRNA levels of ALDH1 and Nodal by Western blot and qRT-PCR assays. The results were analyzed with the clinicopathologic parameters of these patients. The results indicated that expressions of ALDH1 and Nodal were significantly correlated with the differentiation degree, metastasis, number of tumor positive lymph nodes and AJCC stage. ALDH1 was inclined to express more in the worse differentiated degrees, lymph node metastasis, and worse AJCC stage of colorectal cancer patients. And the expression of Nodal was inversely compared with ALDH1.While the expression of ALDH1 was inversely correlated with the Nodal ($r = -0.709$, $P < 0.01$).

Keywords ALDH1 · Nodal · Colorectal cancer · Predictive factors

✉ Bingrong Liu
bingrongliu1963@126.com

[1] Department of Gastroenterology, The Second Affiliated Hospital of Harbin Medical University, Xuefu Road 246, Nangang District, Harbin 150001, Heilongjiang, People's Republic of China

[2] Department of Pathology, Harbin Medical University, Xuefu Road 157, Harbin 150081, People's Republic of China

[3] Department of Infectious Disease, The Second Affiliated Hospital of Harbin Medical University, Xuefu Road 246, Harbin, Heilongjiang 150001, People's Republic of China

Introduction

Colorectal cancer (CRC) ranks highly in terms of mortality worldwide. In Asia, although the incidence of CRC has been decreasing recently, CRC is one of the commonest malignancies with unfavorable prognosis [1]. Colorectal cancer involves in oncogenes and the complicated change of inhibition tumor genes, and it is closely related to the genetic and environmental factors, while the specific reason has not clear. Surgical resection is the most effective treatment of CRC [2]. Therefore, accurate prognostic factors for CRC are important to promote the treatment of CRC patients. To our knowledge, although many reports discovered many valuable prognostic markers of CRC, only few researches pay attention to the prognostic value of combined expressions of ALDH1 and Nodal for CRC patients.

Aldehyde dehydrogenase 1 (ALDH1), a detoxifying enzyme responsible for the oxidation of intracellular aldehydes, and high ALDH1 activity has been showed in human hematopoietic stem cells [3]. Cancers of breast, lung, and prostate also show high ALDH1 activity [4–6]. ALDH1 might be a common marker for both normal and malignant stem cell populations. Since ALDH1 is a specific marker for Cancer initiating cells (CICs) in normal and malignant colon, and is more specific for CICs in the colon than CD44 or CD133 [7].

Nodal is a member of the Transforming Growth Factor Beta (TGF-β) super family, essential in maintaining the pluripotency of human embryonic stem cells. Like most TGF-β signals, Nodal ligands activate serine/threonine kinase receptors that phosphorylate Smad proteins to regulate gene expression [8]. Recent findings have revealed that Nodal was a possible regulator of tumor growth, plasticity and tumor pathogenesis, and holds promise as a new biomarker for metastatic potential [9]. Therefore, studies addressing the role of Nodal in colorectal cancer are meaningful.

As to our knowledge, the prognostic value of combination inspection of ALDH1 and Nodal has not been previously well revealed in CRC. We investigated the protein and messenger RNA (mRNA) expression levels of ALDH1 and Nodal through immunohistochemistry, Western blotting analysis, and quantitative real-time polymerase chain reaction (qRT-PCR). The correlations between ALDH1 and Nodal levels and clinicopathologic parameters were analyzed in CRC tissues.

Materials and Methods

Patients and Tissue Samples

Tissue samples from 108 patients who underwent curative resection for primary or metastatic CRC between April 2013 and May 2014 were obtained from the second affiliated hospital of Harbin Medical University. Match specimens of all patients were frozen and stored in liquid nitrogen for WB and qRT-PCR to assess the ALDH1 and Nodal protein expression and transcript abundance. Clinical and pathological data were obtained, including age, sex, primary tumor location, the presence of lymph node metastasis, the situation of distant metastasis, American Joint Cancer Committee (AJCC) stage, TNM and Dukes stages. According to the World Health Organization Classification of Tumours [10], mucinous adenocarcinoma and signet-ring cell carcinoma conventionally are considered poorly differentiated. Moreover, according to the various primary sites of the cancers, the samples were divided into three group, which were proximal colon, including cecum, ascending colon, transverse colon and splenic flexure; distal colon, including descending colon and sigmoid colon; rectum and juncture of rectum and sigmoid colon [11].

Approval was obtained from the Institutional Review Board of the second affiliated hospital of Harbin Medical University and received ethical approval from the Ethics Committee. All the subjects provided written informed consent, and were assured of anonymity and the confidentiality of patients' data.

Immunohistochemical (IHC) Staining

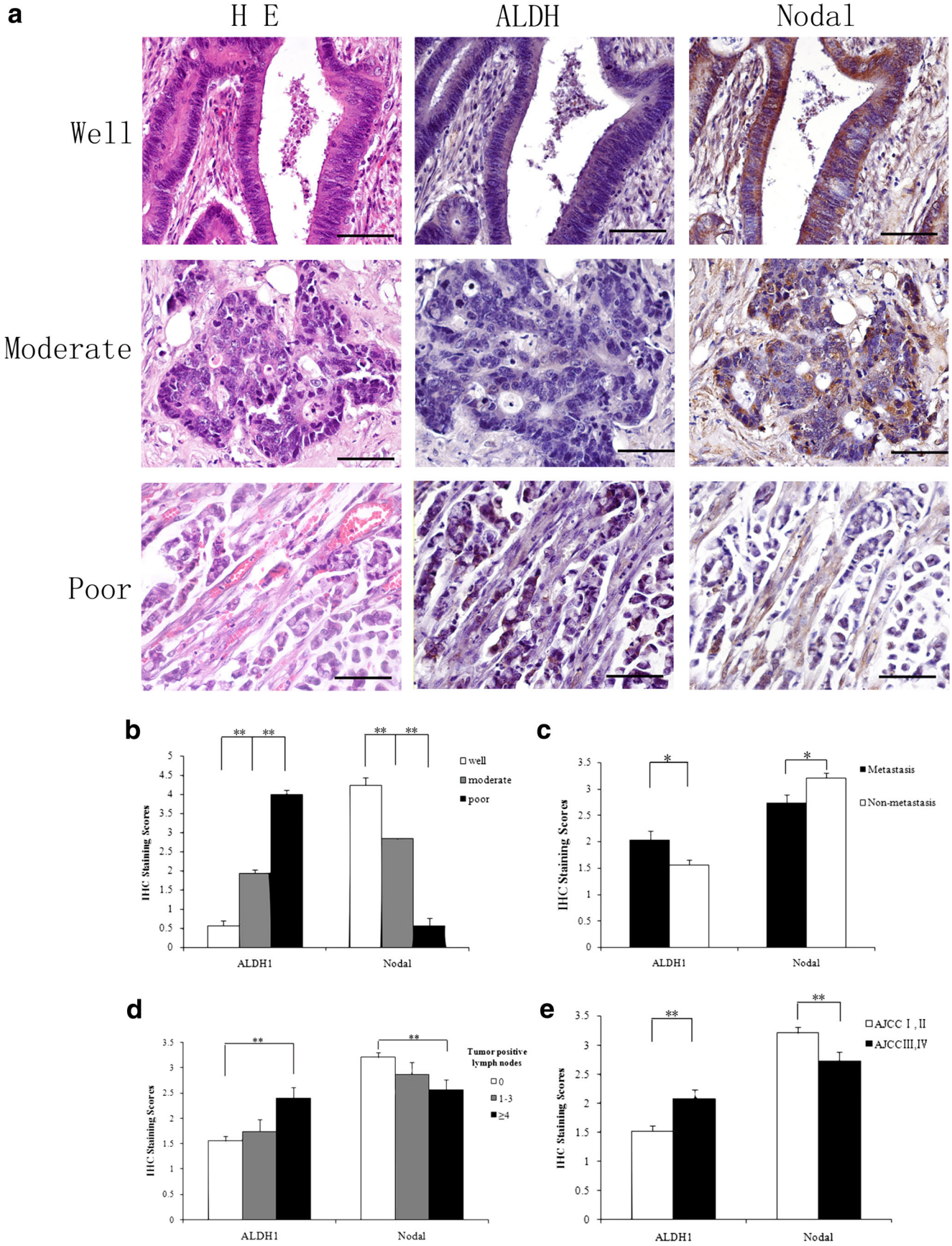
After dewaxing and hydration, the sectioned samples were treated with 3 % H_2O_2 for 5 min to block endogenous peroxidase activity, washed by PBS three times. Subsequently, 10 % normal goat serum (Boster Biological Technology, Wuhan, China, AR0009) was used to block non-specific binding. The sections were incubated with primary antibody, mouse against human ALDH1 (BD, 611, 194) × 100, Nodal (abcam, ab55676) × 100, respectively at 4 °C overnight, washed by PBS three times, followed by incubation with secondary antibody, goat anti-mouse antibodies for 15 min at room temperature, washed by PBS three times. The Polink-1 HRP DAB detection system kit (Zhongshan Jinqiao Biotechnology, Beijing, China, PV6002) was used with 3,3′-Diaminobenzidine tetrahydrochloride kit (Zhongshan Jinqiao Biotechnology, Beijing, China, ALI-9032) as the chromogenic substrate for visualization. Nuclei were counterstained with hematoxylin.

The immunostaining of ALDH1 and Nodal was scored according to the staining intensity and positive cell rate, as follows. The staining intensity: 0 score, absent stained of detecting proteins; 1 score, weak staining showed light yellow; 2 scores, strongly stained with dark yellow or bistre. And the positive cell percentage was graded as follows: 0 point, 0–25 % cells staining positively; 1 point, 25–50 % cells staining positively; 2 points for 50–75 %, and 3 points for 75 % or more respectively. The final results are recorded and judged as the sum of the intensity score and positive cell percentage point of each sample..

Fig. 1 Representative images of expressions of ALDH1 and Nodal in 108 cases of CRC. a Well: well-differentiated CRC; Moderate: moderately differentiated CRC; Poor: poorly differentiated CRC Bar =10 μm, b-e $*P < 0.05$, $**P < 0.01$

Western blot Analysis

Since the number of poorly-differentiated cases is rare, we respectively selected 7 cases of well-differentiated and moderately-differentiated to compare. The total protein contains of the matched tissues of all patient were extracted and suspended in a lysis buffer, which consisted RIPA (Solarbio, Beijing) and PMSF (Solarbio, Beijing). Proteins (25 μg/lane) were fractionated by SDS-PAGE and electrotransferred onto polyvinylidene difluoride membranes, which were then blocked with a blocking buffer consisting of 0.05 % Tween-20 (Invitrogen Life Technologies, Carlsbad, California) and 5 % non-fat powdered milk (Sangon Biotech, Shanghai, China) for 1.5 h, then incubated with a primary antibody at 4 °C overnight. The primary antibodies were same as the ones in IHC: mouse anti-human ALDH1 (1:1000), mouse anti-human Nodal (1:1000) and mouse anti-human GAPDH (1:1000, BD transduction Laboratories). After washed by Tris Buffered Saline with Tween-20, the membrane was incubated with Fluorescein(FITC)-conjugated, affiniPure donkey anti-mouse secondary antibody (Sangon, Shanghai, China) for 1 h, after washing photophobically for 3 times, and antigen-antibody complexes were detected byinfrared imaging system (Odyssey; LI-COR Biosecience, Ltd., Kent, UK).


a
H E
ALDH
Nodal
Well
Moderate
Poor
b
IHC Staining Scores
well
moderate
poor
ALDH1
Nodal
c
IHC Staining Scores
Metastasis
Non-metastasis
ALDH1
Nodal
d
IHC Staining Scores
Tumor positive lymph nodes
0
1-3
≥4
ALDH1
Nodal
e
IHC Staining Scores
AJCC Ⅰ, Ⅱ
AJCC Ⅲ, Ⅳ
ALDH1
Nodal

Reverse Transcription Quantitative Polymerase Chain Reaction (qRT-PCR)

Since the number of poorly-differentiated cases was rare, we respectively selected 7 cases of well-differentiated and moderately-differentiated to compare. Total RNA from the tissue specimens was isolated using RNAsimple Total RNA kit (TIANGEN, Beijing, China) according to the manufacturer's instructions. Complementary DNA was synthesized using the All-in-one™ First-strand cDNA synthesis kit (GeneCopoeia, Carlsbad, California). Quantitative PCR was carried out with 2 μl cDNA in a 20 μl reaction volume using a SGExcel FastSYBR Mixture kit (Sangon Biotech, Shanghai, China) as the manufacturer's specifications. The qRT-PCR was performed on 7500 Fast System (Applied Biosystems, Foster City, CA, USA).The primers used were as follows: ALDH1 (forward, 5′-TCCTGGTTATGGGCCTACAG-3′ and reverse 5′-CTGGCCCTGGTGGTAGAATA-3′); Nodal (forward, 5′-ACCGAGTCCCTTCCACTTGT-3′ and reverse, 5′-CAGAGGCACCCACATTCTTC-3′). The human β-actin gene was used as an endogenous control.

Statistical Analysis

Those data contained three or more groups in each variable were analyzed by Kruskal-Wails H (K) test, and those included two groups in each variable were judged using Mann-Whitney U test, correlation analysis were judged by Spearman test. In all analyses, P<0.05 was considered significant.

Results

The expression of ALDH1 was correlated with the degree of differentiation, metastasis, positive lymph nodes' number, and AJCC stage in CRC.

The staining for ALDH1 was localized in the cytoplasm. Our data showed that the expression level of ALDH1 was more in poorly differentiated (IHC scores, MEAN ± SD, 4 ± 0.6) CRCs than in the well (0.6 ± 0.6) and moderate (1.9 ± 0.7), and it was also more in the moderate than in the well. (P<0.01; Fig. 1a and b). In addition, ALDH1 expressed more in those patients with metastasis in lymph nodes or other organs (2 ± 1.3 v.s. 1.6 ± 0.7; *P*<0.05; Fig. 1c). Compared with the cases that has no tumor positive lymph nodes, the cases that has more tumor positive lymph nodes (≥4) were inclined to express more ALDH1(2.4 ± 1 v.s. 1.6 ± 0.7; P<0.01, Fig. 1d). We also found ALDH1 expressed more in worse AJCC stages (2.1 ± 1.2 v.s. 1.5 ± 0.7; *P*<0.01; Fig. 1e). The WB assays revealed that ALDH1 (55KD) expressed highly in the poorly-differentiated CRCs (Fig. 2b). And qRT-PCR result (Fig. 2a) showed the similar trend, the mRNA level of ALDH1 was more in poorly differentiated CRCs than in the well (*P*<0.05) and moderate (*P*<0.01). The other clinicopathologic parameters, such as patients' sex, age, family history, tumor site and size, invasive depth, and Duke's stage were found no significant correlation with the ALDH1 expression (Table 1). In general, the colorectal adenocarcinoma samples of the poorer differentiation in tumor grade, worse AJCC stages, metastasis that occurred in lymph nodes or other organs were inclined to express more ALDH1.

The expression of Nodal was correlated with the factors of tumor grade, tumor positive lymph nodes' number, metastasis and AJCC stage.

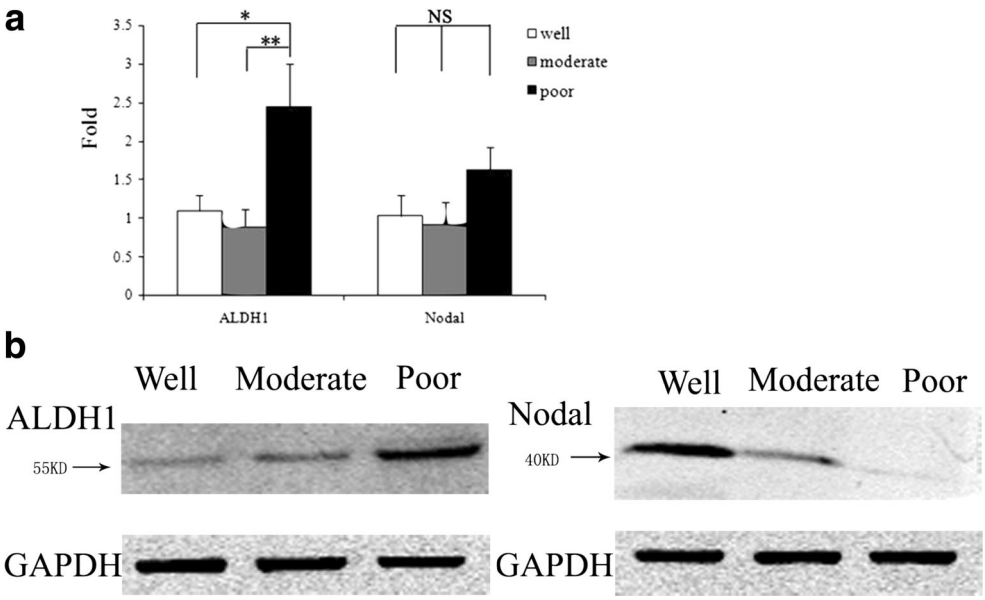


Fig. 2 The mRNA and protein levels of ALDH1 and Nodal in CRC. a *$P < 0.05$, **$P < 0.01$, NS = no significance

Table 1 IHC staining scores of ALDH1 and Nodal in colorectal cancer patients with part of clinical parameters

	n (%)	ALDH1 (mean±SD)	Nodal (mean ± SD)
Sex			
Male	69 (63.9 %)	1.8 ± 1.1	3 ± 1.2
Female	39 (36.1 %)	1.9 ± 1	2.9 ± 0.8
Age, yr			
≤ 45	11 (10.2 %)	1.9 ± 1.4	2.7 ± 1.8
45–65	46 (42.6 %)	1.8 ± 1.2	2.9 ± 0.9
≥ 65	51 (47.2 %)	1.8 ± 0.8	3.1 ± 0.9
Family History			
Yes	9 (8.3 %)	1.7 ± 1.2	3.1 ± 1.2
No	99 (91.7 %)	1.8 ± 1	3 ± 1
Primary site			
Proximal colon[a]	30 (27.8 %)	1.7 ± 1	2.9 ± 1.1
Distal colon[b]	26 (24.1 %)	2 ± 1.2	2.9 ± 1.2
Rectum[c]	52 (48.2 %)	1.8 ± 1	3 ± 1
Tumor size, cm			
< 5	46 (42.6 %)	1.7 ± 1	2.9 ± 1.1
≥ 5	62 (57.4 %)	1.8 ± 1.1	3 ± 1.2
Invasive depth			
Muscular layer	12 (11.1 %)	1.5 ± 0.7	3.5 ± 0.9
Serosa or tunica adventitia	90 (83.3 %)	1.8 ± 1.1	2.9 ± 1.1
over serosa, or to other organs	6 (5.56 %)	2.2 ± 0.4	2.7 ± 0.5
Dukes staging			
A	4 (3.7 %)	1 ± 0.8	3.8 ± 1.3
B	44 (40.7 %)	1.6 ± 0.7	3.1 ± 0.7
C	53 (49.1 %)	2 ± 1.3	2.9 ± 1.3
D	7 (6.5 %)	2.1 ± 0.4	2.6 ± 0.5

Nodal was primarily expressed in the cytoplasm. The expression of Nodal was found higher in the group of well differentiated CRC (4.2 ± 0.6) than in poorly differentiated group (0.6 ± 0.5)and moderate differentiated group (2.8 ± 0.7) of CRC (P<0.01; Fig. 1a and b). And it is more in moderate differentiated group than poorly differentiated group of CRC (*P*<0.01; Fig. 1a and b). In addition, it was found less expressed in those cases with metastasis in lymph nodes or other organs (2.7 ± 1.2 v.s. 3.2 ± 0.8; *P*<0.05; Fig. 1c). It also expressed more in the group of no tumor positive lymph nodes than in the group of more tumor positive lymph nodes (≥4) (3.2 ± 0.8 v.s. 2.6 ± 1; *P*<0.01; Fig. 1d). Furthermore, its expression was less in worse AJCC stages (3.2 ± 0.8 v.s. 2.7 ± 1.2; *P*<0.05; Fig. 1e). The WB assays (Fig. 2b) revealed that expression of Nodal (40KD) was significantly higher in well differentiated groups than poorly differentiated groups of

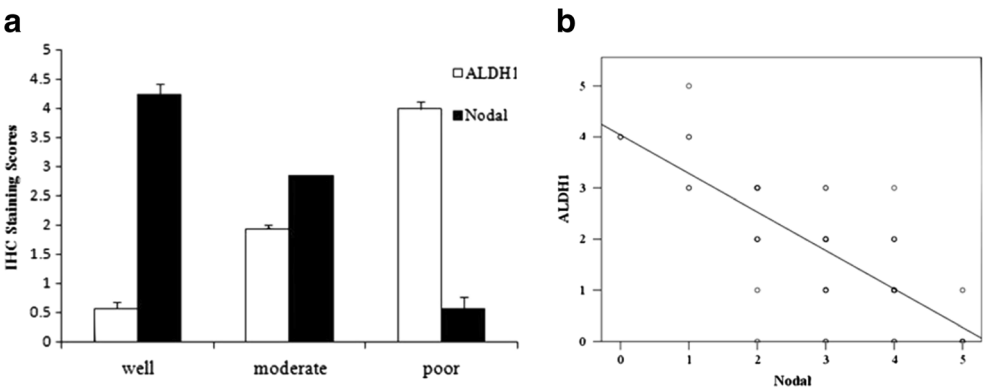


Fig. 3 Relevance of ALDH1 and Nodal expression

CRC. However, by qRT-PCR, we have not found there was statistical significance between the cases of CRC (Fig. 2a). Except these, we found no correlation between the Nodal protein levels and some clinicalpathologic parameters, such as the patient's sex, age, family history, tumor site and size, Duke's stage and invasive depth (Table 1). In general, the colorectal adenocarcinomas groups of well differentiation, negative lymph nodes or organ metastasis, and better AJCC stage were inclined to express more Nodal.

The expression of ALDH1 and Nodal was negatively correlated in CRC.

Interestingly, correlations of the expression levels of ALDH1 and Nodal were found negatively correlated in the CRCs by IHC staining, $r = -0.709$, $P < 0.01$(Fig. 3a and b).

Discussion

In the current study, we were able to identify that the correlation of Nodal, ALDH1 expressions and clinicopathologic parameters in colorectal cancer. To the best of our knowledge, this is the first report demonstrating the relationship of two combined targets as ALDH1 and Nodal and part of clinicopathologic characters of colorectal cancer. We believe that would contribute to elucidating the predictive value of ALDH1 and Nodal for colorectal cancer.

Accompany with the growing interest of cancer stem cell hypothesis, the role of ALDH was accepted as a putative cancer stem cell marker [12]. Due to the ability of irreversibly oxidizing and converting retinaldehyde into retinoic acid, ALDH1 may play an important role of retinoic acid signaling pathway during the differentiation of cells [13]. Based on various analysis, many recent researches are inclined to consider ALDH1 as a poor prognosis factor of colorectal cancer [14–17]. However, there is still remain controversial for specific clinicopathological parameter, such as patients' survival time, grade, lymphatic metastasis which was correlated with the increased expression of ALDH1. Our results showed that there was more ALDH1 expressed in poorly differentiated degree, lower AJCC stage, and more lymphatic metastasis of CRC. All of these indicated ALDH1 was a possible prognostic factor. This is accord with some research results have been described before [18].

Our result also indicated that the expression of Nodal was also correlated with the differential grade, metastasis, lymphatic metastasis and AJCC stages of colorectal cancers. Nodal is secreted factor which belongs to the transforming growth factor beta (TGF-β) superfamily and it plays essential roles in regulating embryonic development and cell fate determinations [19]. TGF-β1 has manifested its versatile role in the breast cancer: in early stages, it shows tumor suppressive effects by inhibiting epithelial cell cycle progression and promotes apoptosis, however, in late stages, it also was linked with increased tumor progression, higher cell motility, cancer invasiveness, and metastasis of breast cancer [20]. Similarly, it has been reported that Nodal played multiple roles, suppressing effect [21, 22] or promoting effect [23, 24], in various kinds of cancer. Our result indicated that Nodal was correlated with the differentiated degree, lymphatic metastasis and AJCC stage of CRC. It may suggest that Nodal could be involved in the process of regulating differentiation of CRC, and it may play a necessary role in providing positional information, such as polarity, for colorectal cancer cells.

Interestingly, we found that the expressions of ALDH1 and Nodal were inversely in CRC. It has been reported that ALDH1 expression was inhibited by TGF-β in gastric carcinoma. And TGF-β signaling may reduce side-population proportion and decreases CICs to inhibit tumorigenesis [25]. Nodal was also reported to inhibit ALDH1 expression by stimulating TGF-β signaling in uterine endometrioid adenocarcinoma [26]. There still remain much research work to reveal the intrinsic mechanism of expressions of ALDH1 and Nodal.

In summary, the expressions of ALDH1 and Nodal are correlated with colorectal cancer differentiation grade, metastasis and AJCC stage. They may become diagnosis markers of colorectal cancer and provide new indicators for assessment of clinical pathological staging of colon cancer. This study attempted to elucidate the relationship of ALDH1 and Nodal expression and prognosis of CRC patients, since it has the shortcoming of a retrospective analysis. However, it was sufficient to evaluate value of CRC with expressions of ALDH1 and Nodal. The Increasing knowledge about multiple roles of ALDH1 and Nodal in carcinogenesis and their potential usefulness in developing anti-cancer immunotherapy should direct more attention to their expressions in cancer.

Conflict of Interest All of the authors declare no conflict of interests.

References

1. Saika K, Sobue T (2013) Cancer statistics in the world. Gan to Kagaku ryoho 40:2475–2480
2. Giuliani F, De Vita F, Colucci G, Pisconti S (2010) Maintenance therapy in colon cancer. Cancer Treat Rev 36(Suppl 3):S42–S45
3. Hess DA, Wirthlin L, Craft TP, Herrbrich PE, Hohm SA, Lahey R, Eades WC, Creer MH, Nolta JA (2006) Selection based on CD133 and high aldehyde dehydrogenase activity isolates long-term reconstituting human hematopoietic stem cells. Blood 107:2162–2169
4. Dave B, Chang J (2009) Treatment resistance in stem cells and breast cancer. J Mammary Gland Biol Neoplasia 14:79–82
5. Jiang F, Qiu Q, Khanna A, Todd NW, Deepak J, Xing L, Wang H, Liu Z, Su Y, Stass SA, Katz RL (2009) Aldehyde dehydrogenase 1 is a tumor stem cell-associated marker in lung cancer. Mol Cancer Res 7:330–338

6. Li T, Su Y, Mei Y, Leng Q, Leng B, Liu Z, Stass SA, Jiang F (2010) ALDH1A1 is a marker for malignant prostate stem cells and predictor of prostate cancer patients' outcome. Lab Investig 90: 234–244
7. Huang EH, Hynes MJ, Zhang T, Ginestier C, Dontu G, Appelman H, Fields JZ, Wicha MS, Boman BM (2009) ALDH1 is a marker for normal and malignant human colonic stem cells and track SC overpolution during cancer tumorigenesis. Cancer Res 69:3382–3389
8. Schier AF, Shen MM (2000) Nodal signalling in vertebrate development. Nature 403:385–389
9. Strizzi L, Hardy KM, Kirschmann DA, Ahrlund-Richter L, Hendrix MJ (2012) Nodal expression and detection in cancer: experience and challenges. Cancer Res 72:1915–1920
10. Hamilton SR, Vogelstein B, Kudo S, Nakamura S, Hainaut P, Rubio AC, Sobin LH, Fogt F, Winawer SJ, Goldgar DE, Jass JR (2000) Carcinoma of the colon and rectum. In: Hamilton SR, Aaltonen LA (eds) World Health Organization Classification of Tumors, International Agency for Research on Cancer (IARC). Press, Lyon, pp. 110–111
11. Li FY, Lai MD (2009) Colorectal cancer, one entity or three. J Zhejiang Univ Sci B 10:219–229
12. Ma I, Allan AL (2011) The role of human aldehyde dehydrogenases in normal and cancer stem cells. Stem Cell Rev 7:292–306
13. Yasmeen R, Jeyakumar SM, Reichert B, Yang F, Ziouzenkova O (2012) The contribution of vitamin a to autocrine regulation of fat depots. Biochim Biophys Acta 1821:190–197
14. Goossens-Beumer IJ, Zeestraten EC, Benard A, Christen T, Reimers MS, Keijzer R, Sier CF, Liefers GJ, Morreau H, Putter H, Vahrmeijer AL, van de Velde CJ, Kuppen PJ (2014) Clinical prognostic value of combined analysis of Aldh1, survivin, and EpCAM expression in colorectal cancer. Br J Cancer 110:2935–2944
15. Fitzgerald TL, Rangan S, Dobbs L, Starr S, Sigounas G (2014) The impact of aldehyde dehydrogenase 1 expression on prognosis for metastatic colon cancer. J Surg Res 192:82–89
16. Oh SY, Sohn SH, Yim H, Lee D, Suh KW, Kim YB (2015) ALDH1 is a prognostic factor for patients treated with neoadjuvant chemoradiotherapy and radical resection for stage III rectal cancer. J Surg Oncol 111:243–247
17. Zhou F, Mu YD, Liang J, Liu ZX, Zhou D, Ning WL, Li YZ, Ding D, Zhang JF (2015) Aldehyde dehydrogenase 1: a specific cancer stem cell marker for human colorectal carcinoma. Mol Med Rep 11: 3894–3899
18. Hou Y, Liu YY, Zhao XK (2013) Expression of aldehyde dehydrogenase 1 in colon cancer. Asian Pac J Trop Med 6:574–577
19. Juan H, Hamada H (2001) Roles of nodal-lefty regulatory loops in embryonic pattering of vertebrates. Genes Cells 6:923–930
20. Zarzynska JM (2014) Two faces of TGF-beta1 in breast cancer. Mediat Inflamm 2014:141747
21. Xu G, Zhong Y, Munir S, Yang BB, Tsang BK, Peng C (2004) Nodal induces apoptosis and inhibits proliferation in human epithelial ovarian cancer cells via activin receptor-like kinase 7. J Clin Endocrinol Metab 89:5523–5534
22. Zhong Y, Xu G, Ye G, Lee D, Modica-Amore J, Peng C (2009) Nodal and activin receptor-like kinase 7 induce apoptosis in human breast cancer cell lines: role of caspase 3. Int J Physiol Pathophysiol Pharmacol 1:83–96
23. De Silva T, Ye G, Liang YY, Fu G, Xu G, Peng C (2012) Nodal promotes glioblastoma cell growth. Front Endocrinol (Lausanne) 3:59
24. Gong Y, Guo Y, Hai Y, Yang H, Liu Y, Yang S, Zhang Z, Ma M, Liu L, Li Z, He Z (2014) Nodal promotes the self-renewal of human colon cancer stem cells via an autocrine manner through Smad2/3signaling pathway. Biomed Res Int 2014:364134
25. Katsuno Y, Ehata S, Yashiro M, Yanagihara K, Hirakawa K, Miyazono K (2012) Coordinated expression of REG4 and aldehyde dehydrogenase 1 regulating tumourigenic capacity of diffuse-type gastric carcinoma-initiating cells is inhibited by TGF-b. J Pathol 228:391–404
26. Wang Y, Jiang Y, Tian T, Hori Y, Wada N, Ikeda J, Morii E (2013) Inhibitory effect of nodal on the expression of aldehyde dehydrogenase 1 in endometrioid adenocarcinoma of uterus. Biochem Biophys Res Commun 440:731–736