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Expression of aldehyde dehydrogenase 1 in colon cancer

Yi Hou^{1Δ}, Yi-Yi Liu^{2Δ}, Xiao-Kun Zhao^{1*}¹Department of Surgery, the Second Xiang-ya Hospital, Central South University, Changsha, Hunan 410011, China²Department of Surgery, the Third Hospital of Yueyang, Yueyang, Hunan 414000, China

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ABSTRACT

Objective: To study the expression of ALDH1 in colon cancer and its clinical significance.**Methods:** The expression of ALDH1 was examined in 98 surgical specimens of primary colonic carcinoma and 15 normal colon tissues with immunohistochemistry method. The correlations of the expression with clinicopathological parameters and prognosis of colon cancer were analyzed.**Results:** The positive rate of expression of ALDH1 was 76.5% (75/98) in the cancer tissues and 13.3% (2/15) in normal colon tissues. There were an obvious statistical difference ($P < 0.05$) between the two groups. The ALDH1 expression was significantly correlated with the histological grade, TNM stages and lymph node metastasis in colon cancer ($P < 0.05$). It was also related with patients' survival time, those with positive expressions had a poor prognosis ($P < 0.05$). **Conclusions:** The results suggest that the overexpression of ALDH1 plays important roles in proliferation and progression in colon cancer, the ALDH1 may be a valuable marker to predict the biological behavior and trend of metastasis of colon cancer.

1. Introduction

Colon cancer is one of the common malignancy and is the third commonest gastrointestinal tumors. The incidence of 40 to 60-year-old people is the highest and the male to female ratio is 2–3:1. According to the world epidemiological survey it is found that the highest incidence of colon cancer is in Europe, the United States and the other places, ranking the second commonest visceral tumor. Colon cancer involves in oncogenes and the complex change of inhibition tumor genes, and it is closely related to the genetic and environmental factors, while the specific reason has been not yet very clear. Surgical resection is the most effective treatment of colon cancer [1].

Aldehyde dehydrogenase is a group of the isoenzyme which can oxidize acetaldehyde into acetic acid and protect

cells from peroxides damage [2]. In recent years, the aldehyde dehydrogenase (ALDH1) gene has become the one of the research focus of the tumor development mechanism. The foreign study found that ALDH1 is one of the indicators to identify colon cancer stem cells [3], but there is no reported on ALDH1 related to colon cancer research in domestic currently. In this study, ALDH1 protein expression was detected by immunohistochemical methods in tumor tissue of 98 patients with colon cancer and the tumor adjacent tissues of 15 patients, and the relationship was analyzed between the ALDH1 protein expression with clinic pathological characteristics and prognosis.

2. Materials and methods

2.1. Clinical data

The specimens from patients under hospitalization and surgery were selected by pathological diagnosis from March 2005 to August 2006, including cancer tissue from 98 patient with colon cancer and tumor adjacent tissues with 8 cm

*Corresponding author: Xiao-Kun Zhao, Department of Surgery, the Second Xiang-ya Hospital, Central South University, Changsha, Hunan 410011, China.
Tel: +86 13808469229

E-mail: xiaokunzhao2002@yahoo.com.cn

ΔThese authors contributed equally to this work.

tumor margin at organizations from another 15 patient. A total of 98 patients with colon cancer included 65 males and 33 females, aged from 34 to 76 years old (average age 55.2 years old). They did not receive anticancer drug treatment before surgery and they had complete clinical data and pathological data. The clinical pathological stage were according to TNM stage of the international developed by Union against Cancer.

2.2. Main reagents and experimental methods

ALDH1 polyclonal antibody was from BD Biosciences, diluted 1:100. Immunohistochemical staining kit and DAB kit was from American VECTOR LAB Company. The slices were placed at a 65 °C oven and dewaxed, then were taken to 95 °C 1 × sodium citrate and antigen retrieval in 60 min. The specimens was closed with goat serum from immunohistochemical staining kit, and ALDH1 antibody diluted was added with PBS, stayed overnight at 4 °C refrigerator; while the specimens from negative control group were added with PBS instead of the first antibody. The next day it was added by secondary antibody, horseradish peroxidase–labeled streptavidin, then was incubated, stained by using DAB color rendering, and hematoxylin counter–stain.

2.3. Judgment of immunohistochemical staining

ALDH1 was located in the cytoplasm, stained light yellow to tan under the microscope. A total of 20 vision was randomly selected, 100 to 200 cancer cells was counted under 400× Light microscope. The positive cytoplasm was yellow, the percentage of positive cells was calculated and staining results was determined with two scoring method. Positive cell count: <5%, 0 point; 5%–25%, 1 point; 26%–50%, 2 points; 51%–75%, 3 points; > 75%, 4 points. The classification of staining intensity by cytoplasmic dying: free yellow dye was 0 point; yellowish 1 point; yellow or deep yellow 2 points; brown or tan 3 points. Both scored multiplied was greater than 1 for the ALDH1 positive staining[4].

2.4. Statistical analysis

The differences in ALDH1 expression of each group compared with the *Chi*–square test or Fisher’s exact test. The survival curve was drawn by Kaplan–Meier method and the survival rate compared by Log–Rank test. All the data were analyzed by SPSS15.0 statistical software, $P < 0.05$ was considered statistically significant.

3. Results

3.1. ALDH1 expression in colon cancer and cancer adjacent tissues

ALDH1 protein positive expression was observed in 75 cases (76.5%) out of 98 cases with colon cancer tissue and those of negative expression was in 23 cases (23.5%), while ALDH1 protein positive expression in the adjacent tissues was 2 cases (13.3%), and those of negative expression was 13 cases (86.7%) (Figure 1). ALDH1 protein expression was significant differences between the cancer tissue and cancer adjacent tissues ($\chi^2 = 23.933$, $P < 0.05$)

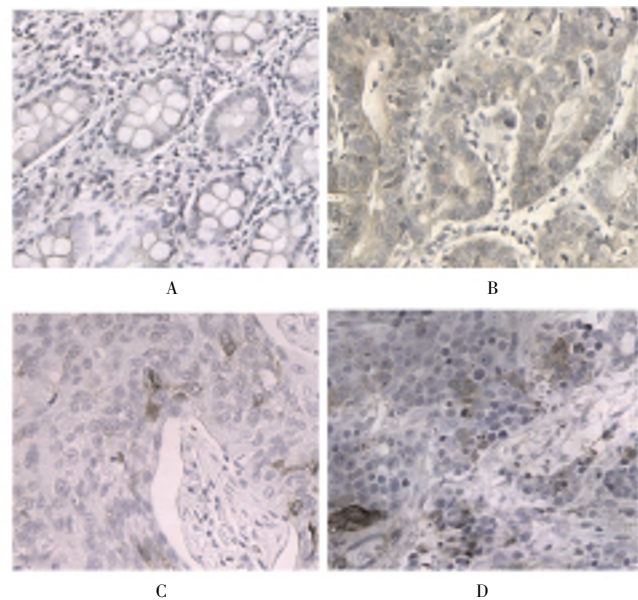


Figure 1. ALDH1 expression in the colon cancer and cancer adjacent tissue by immunohistochemical assay (200–fold).

A Tumor adjacent tissues; B High–differentiated colon adenocarcinoma (I grade); C Moderately differentiated colon adenocarcinoma (II grade); D Poorly differentiated colon adenocarcinoma (III grade).

3.2. Relationship between ALDH1 protein expression and clinic pathological factor in colon cancer

ALDH1 positive expression rate was significantly different among the poorly, moderate and high differentiated colon adenocarcinoma. In colon cancer, ALDH1 positive expression rate of patients with lymph node metastasis was significantly higher than the group without lymph node metastasis. ALDH1 positive expression rate of colon cancer III + IV by TNM staging of stage was significantly higher than that of I + II stage, the differences were statistically significant ($P < 0.05$). It showed that ALDH1 expression of colon cancer tissue was related with the differentiation, lymph node metastasis and clinical pathological staging.

Table 1

Relationship between ALDH1 protein expression and clinic pathological factor in colon cancer.

Clinic pathological factors	<i>n</i>	ALDH1 protein expression		χ^2	<i>P</i>	
		Positive	Negative			
Age	>60	45	34 (75.6%)	11 (24.4%)	0.044	0.834
	<60	53	41 (77.3%)	12 (22.7%)		
Sex	Male	65	50 (76.9%)	15 (23.1%)	0.017	0.898
	Female	33	25 (75.8%)	8 (24.2%)		
Differentiation degree	Highly differentiated	35	22 (62.9%)	13 (37.1%)	6.551	0.038
	Moderately differentiated	22	17 (77.3%)	5 (22.7%)		
	Poorly differentiated	41	36 (87.8%)	5 (12.2%)		
Lymph node metastasis	I + II	35	22 (62.8%)	13 (37.2%)	5.667	0.017
	III + IV	63	53 (84.1%)	10 (15.9%)		
TNM staging	I + II	35	22 (62.8%)	13 (37.2%)	5.667	0.017
	III + IV	63	53 (84.1%)	10 (15.9%)		

3.3. ALDH1 protein expression and prognosis in colon cancer

Follow-up of 98 patients showed 31 cases died and 6 cases were lost. The overall average survival time was 1 693 days, 1-year survival rate was 94.9%, 5-year survival rate was 68.4% by the Kaplan–Meier method. In ALDH1-positive group, the average survival time was 1 495 days, one year survival rate was 94.7% and 5-year survival rate was 65.3%. In ALDH1 negative group, the average survival time of 1 991 days, one year survival rate was 95.7%, 5-year survival rate of 78.2%. Survival curves of the two groups showed prognosis of patients in ALDH1 negative group was significantly better than that of positive group (Log.Rank test, $P = 0.020$) (Figure 2).

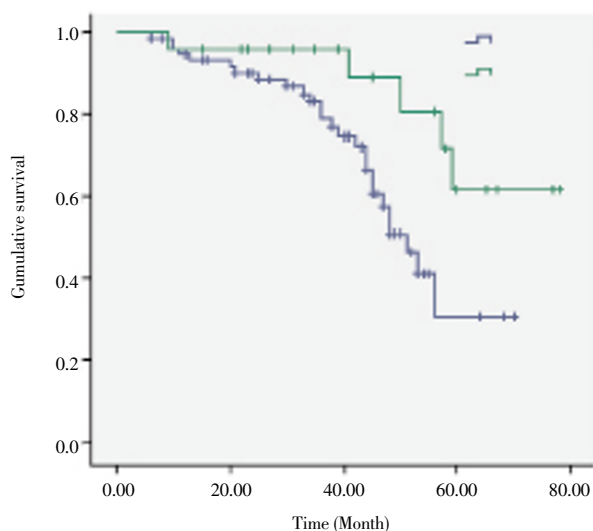


Figure 2. Survival curves of ALDH1 positive and negative expression in colon cancer tissue.

4. Discussion

ALDH is a set of isozymes which can oxidized acetaldehyde into acetic acid and avoid the oxidation of acetaldehyde into acetic acid by aldehyde peroxide damage cells, and it is highly expressed in hematopoietic stem cells[5]. ALDH1 protein expression is in the cytoplasm, its gene is located on chromosome 9, containing 13 exons and encoding a 501 amino acid residues. Its molecule is composed of two subunits, one of which located in the enzyme active site, and the other for the stable of quaternary structure. Under the conditions the coenzyme I exist, It catalyzes the dehydrogenation reaction of alcohols, aldehydes and ketones, and off the hydrogen were accepted by the NAD, becoming NADPH I . It catalytic dehydrogenation reaction speed for butylaldehyde, cinnamic aldehyde, benzaldehyde were more than for acetaldehyde. It can catalytic including ethanol, some of the primary or secondary alcohols, aldehydes and ketones of the dehydrogenation reaction, the catalytic *n*-butylaldehyde, cinnamaldehyde, benzaldehyde dehydrogenation reaction speed ratio acetaldehyde off the hydrogen is accepted by the NAD, become reduced coenzyme I . Studies have shown that ALDH1 plays an important role in the differentiation of hematopoietic stem cell[6]. In recent years researchers screen hematopoietic stem cell subsets by ALDH activity[7]. Huang *et al*[3] found that ALDH1 protein expression was in the basal cells of the normal colonic glands. ALDH1 positive cells of colon cancer increased significantly, and ALDH1 expression of positive tumor cells with the characteristics of stem cells, which have similar stem cells potential with self renew and differentiation.

A number of studies showed that the ALDH1 protein is

highly expressed in a variety of tumor tissue of the organ, such as lung cancer, prostate cancer, breast cancer, bladder cancer^[8–12]. And ALDH1 high expression is closely related to tumor occurrence, development, deterioration and prognosis. In this study we found ALDH1 protein expression were significant different between colon carcinoma and paracancerous organizations ($\chi^2 = 23.933$, $P = 0.000$), and they were significant different among the poorly, moderate and high differentiated colon carcinoma tissue ($\chi^2 = 6.551$, $P = 0.038$). It showed that upregulation of ALDH1 protein was related to the colon cancer. Colon tissue varied from normal gland to invasive cancer with an increasing level of ALDH1 protein expression. ALDH1 protein plays a very important role in colon cancer development and infiltration process. So it can be a valuable marker in clinical detection of colon cancer early lesions and the development process of the disease.

At present, the relations between ALDH1 protein expression and clinic pathological factors for colon cancer at home and abroad have not been reported. In this study, we found ALDH1 protein expression was relevant to colon cancer tissue differentiation, lymph node metastasis and clinic pathological staging ($P < 0.05$). Protein levels showed that ALDH1 expression was related to the invasion and transfer of the colon cancer. ALDH1 expression is an important indicator for the judgment of clinical diagnosis and prognosis of colon.

Experiments showed that ALDH1 protein expression was related to long-term survival of many tumors such as lung cancer, breast cancer, prostate cancer^[8,9,11]. In this study, survival rate was statistically significant different between ALDH1 protein positive patients and negative patients ($P = 0.020$). It showed that ALDH1 expression in colon cancer tissue was related with the patient's survival rate. The above results showed that ALDH1 expression of colon cancer was related to the occurrence and development. The results suggested that inhibiting the expression of ALDH1 protein could reduce colon cancer growth, invasion and metastasis ability to improve the survival rate of patients.

In summary ALDH1 protein is related with colon cancer growth, invasion and metastasis. It may become a diagnosis marker of colon cancer and provide a new basis and indicators for the early diagnosis, prognostic assessment of clinical pathological staging of the colon cancer. But the mechanism of ALDH1 protein in colon cancer development is not yet clear. At the same time, the intervention of ALDH1 may become a new treatment for colon cancer.

Conflict of interest statement

We declare that we have no conflict of interest.

References

- [1] Giuliani F, De Vita F, Colucci G, Pisconti S. Maintenance therapy in colon cancer. *Cancer Treat Rev* 2010; **36**(3): S42–S45.
- [2] Yoshida A, Hsu LC, Dave V. Retinal oxidation activity and biological role of human cytosolic aldehyde dehydrogenase. *Enzyme* 1992; **46**: 239–244.
- [3] Huang EH, Hynes MJ, Zhang T, Ginestier C, Dontu G, Appelman H, et al. Aldehyde dehydrogenase 1 is a marker for normal and malignant human colonic stem cells (SC) and tracks SC overpopulation during colon tumorigenesis. *Cancer Res* 2009; **69**(8): 3382–3389.
- [4] Luo J, Zha S, Gage WR, Dunn TA, Hicks JL, Bennett CJ, et al. Alpha-methylacyl-CoA racemase: a new molecular marker for prostate cancer. *Cancer Res* 2002; **63**: 2220–2226.
- [5] Hohm S, Zhou P, Eades WC, Hess DA, Craft TP, Wirthlin L, et al. Widespread non-hematopoietic tissue distribution by transplanted human progenitor cells with high aldehyde dehydrogenase activity. *Stem Cells* 2006; **26**: 611–620.
- [6] Chute JP, Muramoto GG, Whitesides J, Colvin M, Safi R, Chao NJ, et al. Inhibition of aldehyde dehydrogenase and retinoid signaling induces the expansion of human hematopoietic stem cells. *Proc Natl Acad Sci USA* 2006; **103**: 11707–11712.
- [7] Fallon P, Gentry T, Baller AE. Mobilized peripheral blood SSC loALDHbr cells have the phenotypic and functional properties of primitive haematopoietic cells and their number correlates with engraftment following autologous transplantation. *Br J Haematol* 2003; **122**(1): 99–108.
- [8] Li T, Su Y, Mei Y, Leng Q, Leng B, Liu Z, et al. ALDH1A1 is a marker for malignant prostate stem cells and predictor of prostate cancer patients' outcome. *Laboratory Invest* 2010; **90**: 234–244.
- [9] Jiang F, Qiu Q, Khanna A, Todd NW, Deepak J, Xing L, et al. Aldehyde dehydrogenase 1 is a tumor stem cell-associated marker in lung cancer. *Mol Cancer Res* 2009; **7**(3): 330–338.
- [10] Su Y, Qiu Q, Zhang X. Aldehyde dehydrogenase 1A1-positive cell population is enriched in tumor-initiating cells and associated with progression of bladder cancer. *Cancer Epidemiol Biomarkers Prev* 2010; **19**(2): 327–337.
- [11] Zhou L, Yu P, Wang JF. Aldehyde dehydrogenase 1 protein expression in breast cancer and its clinical significance. *Tumor* 2009; **29**(7): 663–667.
- [12] Han ML, Wu CY, Wang YM. Aldehyde dehydrogenase protein and breast cancer. *Fudan Univ J* 2010; **37**(6): 747–749.