Advances in Modern Oncology Research

doi: 10.18282/amor.v1.i2.15

ORIGINAL RESEARCH ARTICLE

The correlation between aldehyde deydrogenase-1A1 level and tumor shrinkage after preoperative chemoradiation in locally advanced rectal cancer

Rhandyka Rafli[1*], Soehartati A Gondhowiardjo[1], Adang Bachtiar Kantaatmadja[2], Sahat Matondang[3], Ening Krisnuhoni[4]

[1]Department of Radiotherapy, University of Indonesia, Jakarta, Indonesia
[2]Department of Public Health, University of Indonesia, Jakarta, Indonesia
[3]Department of Radiology, University of Indonesia, Jakarta, Indonesia
[4]Department of Anatomical Pathology, Cipto Mangunkusumo Hospital, Jakarta, Indonesia

***Abstract*:** This study was performed to determine the correlation between aldehyde dehydrogenase-1A1 (ALDH1A1) level and tumor shrinkage after chemoradiation in locally advanced rectal cancer. This is a retrospective study of 14 locally advanced rectal cancer patients with long course neoadjuvant chemoradiation. The ALDH1A1 level was measured using ELISA from paraffin embedded tissue. Tumor shrinkage was measured from computed tomography (CT) scan or magnetic resonance imaging (MRI) based on Response Evaluation Criteria in Solid Tumor v1.1 (RECIST v1.1). The mean of ALDH1A1 level was 9.014 ± 3.3 pg/mL and the mean of tumor shrinkage was 7.89 ± 35.7%. Partial response proportion was 28.6%, stable disease proportion was 50% and progressive disease proportion was 21.4%. There was a significantly strong negative correlation (r = –0.890, p < 0.001) between ALDH1A1 and tumor shrinkage. In conclusion, tumor shrinkage in locally advanced rectal cancer after preoperative chemoradiation was influenced by ALDH1A1 level. Higher level of ALDH1A1 suggests decreased tumor shrinkage after preoperative chemoradiation.

***Keywords*:** ALDH1A1; rectal cancer; chemoradiation; RECIST v1.1

***Citation*:** Rafli R, Gondhowiardjo SA, Kantaatmadja AB, et al. The correlation between aldehyde deydrogenase-1A1 level and tumor shrinkage after preoperative chemoradiation in locally advanced rectal cancer. Adv Mod Oncol Res 2015; 1(2): 112–116; http://dx.doi.org/10.18282/amor.v1.i2.15.

*Correspondence to: Rhandyka Rafli, Department of Radiotherapy, University of Indonesia, Jakarta, 3173030, Indonesia, bubuyrafli@gmail.com

Received: 31st July 2015; **Accepted:** 19th October 2015; **Published Online:** 2nd December 2015

Rectal cancer is the third most common malignancy in the world and also in Indonesia. Worldwide, there are 1.2 million new cases of colorectal cancer diagnosed annually with 600,000 death cases each year[1]. Chemoradiation as neoadjuvant therapy before surgery to shrink tumor size is widely accepted as a treatment for locally advanced rectal cancer.

Radiation exposure to rectal cancer cell will increase reactive oxygen species that can alter membrane bilayer and cause lipid peroxidation. The breakdown products of lipid peroxides are mostly aldehydes, which may serve as "oxidative stress second messengers" with prolonged half-life and the ability to diffuse from their site of formation to nucleus, compared to reactive oxygen species (ROS). Cells possess aldehyde dehydrogenase-1 (ALDH1), a family of polyform enzyme, which detoxifies these aldehydes. ALDH1 is located in cytoplasm, mitochondria or nucleus[2-4].

In addition to its known function to oxidize aldehyde, ALDH1 was also found in cancer stem cell (CSC). In 2007, Ginestieret and co-workers have successfully demonstrated the first isoform of ALDH1 as a marker for

normal and malignant human mammary stem cell and predictor of poor clinical outcome[5]. In the following years, ALDH1 activity would be used successfully as a CSC marker for many cancers including lung, liver, bone, colon, pancreatic, prostate, head and neck, bladder, thyroid, brain, melanoma and cervical[6]. With the exception of one recent study on malignant melanoma, increasing evidence suggests that ALDH1 activity is a universal CSC marker[7].

The role of ALDH1 in rectal cancer is not fully understood. Avoranta et al. have demonstrated the prognostic value of ALDH1 in early rectal cancer that received postoperative adjuvant chemotherapy regimens through immunohistochemistry[8]. This study was in line with other colorectal studies, showing the ALDH1 correlation with 5-FU plus oxaliplatin (FOLFOX) resistance in colorectal cancer[9].

One of the isoforms of ALDH1, such as ALDH1A1, was involved in retinoic acid (RA) cell signaling via RA production by oxidizing all-*trans*-retinal and 9-*cis*-retinal. Depending on cellular context, this may lead to cell proliferation, apoptosis and cell cycle arrest. This function, in particular, has been linked to the "stemness" characteristic of cancer stem cell and through a different mechanism, cancer stem cell inherently became more resistant to chemo and radiotherapy[6,10,11].

These properties are believed to hold an important role in tumor response after chemoradiation. Several qualitative and semi-quantitative studies using immunohistochemistry showed that the expression of ALDH1A1 in cell cytoplasm have no prognostic significance in rectal cancer, but the expression of ALDH1A1 in stromal and the nucleus is associated with shorter survival[12].

In order to provide additional analysis of the biological relevance of ALDH1A1 in locally advanced rectal cancer, we examined the level of ALDH1A1 using the quantitative method with enzyme-linked immunosorbent assay (ELISA) to determine the correlation between ALDH1A1 level in rectal cancer tissue and tumor shrinkage after preoperative chemoradiation.

Materials and methods

Study design

This retrospective study enrolled locally advanced rectal cancer patients (T3–4 N0/+ M0) who underwent radiation therapy in Cipto Mangunkusumo Hospital from January 2009 to January 2014. Out of 144 patients, only 43 patients were able to complete the long course chemoradiation (46–50 Gy in 23–25 fractions) with concurrent Capecitabine or FOLFOX. Also, only 14 from 43 patients who had a decent formalin fixed-paraffin embedded (FFPE) rectal cancer tissue, base and evaluation CT-scan or magnetic resonance imaging (MRI) were found to be eligible for this study. Only three patients underwent surgery after chemoradiation.

Deparaffinization and protein extraction

The study material consisted of FFPE tissue from a biopsy sample. A section with 25 μm thickness and area 40–100 mm^2 were cut from FFPE blocks. Paraffin was removed by washing with xylene 3 times, followed by serial washing with 100%, 70%, and 40% alcohol and aqua dest mixtures. Tissue was rinsed with phosphate buffered saline (PBS), homogenized in 1:1 PBS and stored overnight at –20 ℃. After two cycles, freeze-thaw cycles were performed to break the cell membranes. The homogenates were centrifuged for 5 min at 5000 × *g*, in 2–8 ℃. The supernatant was removed and assayed immediately.

Enzyme-linked immunosorbent assay

ALDH1A1 concentration was determined by using Cusabio human retinal dehydrogenase 1 (ALDH1A1). 100 μL of sample supernatant and provided standard were added to each well and incubated for 2 h at 37 ℃. Subsequently, the liquid layer in each well was removed, and 100 μL biotin antibody were added to each well. The mixture was incubated for 1 h at 37 ℃. Wells were aspirated and washed 3 times using washing buffer. 100 μL HRP-avidin were added to each well and incubated for 1 h at 37 ℃. After removing the liquid and 5 times of washings with washing buffer, 90 μL tetramethylbenzidine (TMB) substrate were added to each well and incubated for 15–30 min at 37 ℃. Then, 50 μL of stop solution were added to each well. Within 5 min, ELISA plates were read in a microplate reader, which was set to 450 nm.

Tumor shrinkage evaluation

Baseline imaging and evaluation after chemoradiation were collected by 5 mm slice CT scan or MRI. The longest tumor diameter from baseline and evaluation were compared and classified using RECIST v1.1 methods[13]. Imaging baseline was done within 4 weeks before chemoradiation, and imaging evaluation was done after 4 weeks upon completion of chemoradiation. The longest diameter of tumor was measured and compared with the baseline and evaluation imaging. Correlation between ALDH1A1 level and tumor shrinkage was analyzed using bivariate (Pearson) correlation with bootstrap.

doi: 10.18282/amor.v1.i2.15

Ethics statement

This study was approved by the ethics committee of Medical Faculty, University of Indonesia.

Results

Patient characteristics and outcome

A total of 14 patients were included in our analysis. After preoperative chemoradiation, 4 patients (28.6%) had partial response, 7 patients (50%) with stable disease and 3 patients with progressive disease (*Table 1*).

Table 1 Characteristics of patients treated with neoadjuvant chemoradiation for rectal cancer

		N	%
Number of patients		14	
Gender	: Male/Female	6/8	43/57
T Stage (before treatment)	: T3	2.0	14.3
	: T4	12.0	85.7
N Stage (before treatment)	: N0	2.0	14.3
	: N1	7.0	50.0
	: N2	5.0	35.7
Age	: Mean (SD)	46.8	12.9
Karnofsky performance status	: ≥80	11.0	78.6
	: <80	3.0	21.4
Pathology	: Adenocarcinoma	12.0	71.4
	: Signet ring cell	2.0	28.6
Chemotherapy	: Capecitabine	10.0	71.4
	: FOLFOX	4.0	28.6
Response after chemoradiation	: Partial response	4.0	28.6
	: Stable disease	7.0	50.0
	: Progressive disease	3.0	21.4

ALDH1A1 and tumor shrinkage

Mean of ALDH1A1 level was 9.014 pg/mL. The mean of the longest tumor diameter before chemoradiation was 4.42 cm and after chemoradiation was 4.09 cm. The mean of tumor shrinkage percentage was 7.89%. There was a significantly strong negative correlation (r = –0.890, p < 0.001) (*Figure 1*) with higher ALDH1A1 indicating a decreased tumor shrinkage response (*Tables 2 and 3*).

Table 2 ALDH1A1 results and tumor size and shrinkage after chemoradiation

	Mean
ALDH1A1	9.014 ± 3.3 pg/mL
Tumor largest diameter before chemoradiation	4.42 ± 1.33 cm
Tumor largest diameter after chemoradiation	4.09 ± 1.99 cm
Tumor shrinkage percentage	7.89 ± 35.7 %

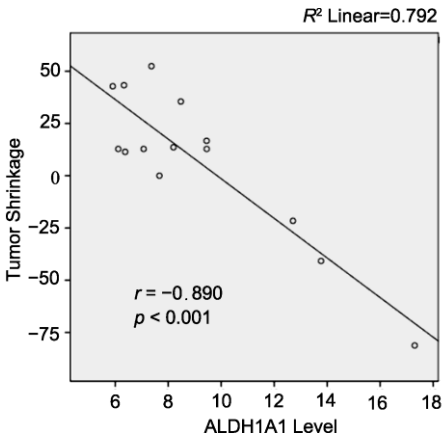

Figure 1 ALDH1A1 level has a significantly strong negative correlation with tumor shrinkage after chemoradiation. r = –0.890 and p < 0.001

Table 3 Tumor shrinkage and ALDH1A1 level in tumor tissue

No	Tumor diameter (cm)		% Shrinkage	RECIST	ALDH1A1 (pg/mL)
	Before chemoradiation	After chemoradiation			
1	7.0	4.0	42.8	PR	5,906
2	4.4	3.8	13.6	SD	8,198
3	3.0	1.7	43.3	PR	6,332
4	4.2	2.0	52.4	PR	7,368
5	2.9	2.9	0.0	SD	7,668
6	3.9	3.4	12.8	SD	7,074
7	4.8	8.7	–81.2	PD	17,306
8	4.9	6.9	–40.8	PD	13,770
9	7.0	6.1	12.8	SD	6,115
10	3.7	4.5	–21.6	PD	12,708
11	3.5	3.1	11.4	SD	6,376
12	3.9	3.4	12.8	SD	9,453
13	5.6	4.7	16.7	SD	9,450
14	3.1	2.0	35.5	PR	8,477

Abbreviations: PR = Partial response; SD = Stable disease; PD = Progressive disease

doi: 10.18282/amor.v1.i2.15

Discussion

This is a quantitative study using ELISA to determine ALDH1A1 level from FFPE rectal cancer tissues. Previous ALDH1 studies on rectal cancer mostly used a semi-quantitative method such as immunohistochemistry. The tumor shrinkage was evaluated using RECIST v1.1, which is widely used as a tool to evaluate solid tumor response. The pathological response was unable to be assessed due to low number of operation after chemoradiation.

ALDH1A1 has been proposed in association with worse prognosis and response to chemotherapy[12]. The expression of ALDH1A1 in normal crypts and colorectal carcinoma tissues has been previously investigated. Researchers have indicated that cells with ALDH1A1 expression were sparse and limited to the bottom of the normal crypts, where the stem cells or the proliferative cells reside[14]. ALDH1A1 is also known for its capability to differentiate stem cell cancer and non-stem cell cancer[15].

Xu et al.[16] stated the different results with heterogeneous pattern of ALDH1A1 staining between rectal cancer cell and adjacent stromal cell. 32.3% of the samples showed a high expression in cancer cell and low expression in adjacent stromal cell. 48.8% of the samples showed a high expression of ALDH1A1 in adjacent stromal cell. Only 16% showed no difference from cancer cell and adjacent stromal[16].

Our present study measured the quantitative level of ALDH1A1 from rectal cancer biopsy tissue that contains both cancer cell and adjacent stromal tissue. It was also shown that there was a strong negative and significant correlation between ALDH1A1 level in FFPE rectal cancer tissue with tumor shrinkage after chemoradiation.

Chemoradiation response is lower (28.6%) compared to the previous study by Lim et al. (40.8%)[17], which may be due to a fewer number of samples in this study. There is a prospect of using ALDH1A1 as a tool to select more suitable locally advanced rectal patients who will undergo preoperative chemoradiation to avoid unnecessary morbidity. A further study with larger sample size is needed to validate it.

Conclusion

ALDH1A1 level in locally advanced rectal cancer tissue is associated with decreased tumor shrinkage response after preoperative chemoradiation.

Conflict of interest

The authors declared no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

References

1. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC Cancer Base. No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer. 2013. Available from: http://globocan.iarc.f.
2. Barrera G. Oxidative stress and lipid peroxidation products in cancer progression and therapy. ISRN Oncol 2012; 2012: 137289.
3. Vasiliou V, Nebert DW. Analysis and update of the human aldehyde dehydrogenase (ALDH) gene family. Hum Genomics 2005; 2(2): 138–143.
4. Lindahl R. Aldehyde dehydrogenases and their role in carcinogenesis. Crit Rev Biochem Mol Bio 1992; 27(4-5): 283–335.
5. Ginestier C, Hur MH, Charafe-Jauffret E, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell 2007; 1(5): 555–567.
6. Marcato P, Dean CA, Giacomantonio CA, et al. Aldehyde dehydrogenase: its role as a cancer stem cell marker comes down to the specific isoform. Cell Cycle 2011; 10(9): 1378–1384.
7. Prasmickaite L, Engesæter BØ, Skrbo N, et al. Aldehyde dehydrogenase (ALDH) activity does not select for cells with enhanced aggressive properties in malignant melanoma. PLoS One 2010; 5(5): e10731.
8. Avoranta ST, Korkeila EA, Ristamaki RH, et al. ALDH1 expression indicates chemotherapy resistance and poor outcome in node-negative rectal cancer. Hum Pathol 2013; 44(6): 966–974.
9. Oh PS, Patel VB, Sanders MA, et al. Schlafen-3 decreases cancer stem cell marker expression and autocrine/ juxtacrine signaling in FOLFOX-resistant colon cancer cells. Am J Physiol Gastrointest Liver Physiol 2011; 301(2): G347–G355.
10. Vogler T, Kriegl L, Horst D, et al. The expression pattern of aldehyde dehydrogenase 1 (ALDH1) is an independent prognostic marker for low survival in colorectal tumors. Exp Mol Pathol 2012; 92(1):111–117.
11. Ishii H, Iwatsuki M, Ieta K, et al. Cancer stem cells and

doi: 10.18282/amor.v1.i2.15

chemoradiation resistance. Cancer Sci 2008; 99(10): 1871–1877.

12. Kahlert C, Gaitzsch E, Steinert G, et al. Expression analysis of aldehyde dehydrogenase 1A1 (ALDH1A1) in colon and rectal cancer in association with prognosis and response to chemotherapy. Ann Surg Oncol 2012; 19(13): 4193–4201.
13. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 2009; 45(2): 228–247.
14. Hessman CJ, Bubbers EJ, Billingsley KG, et al. Loss of expression of the cancer stem cell marker aldehyde dehydrogenase 1 correlates with advanced-stage colorectal cancer. Am J Surg 2012; 203(5): 649–653.
15. Balicki D. Moving forward in human mammary stem cell biology and breast cancer prognostication using ALDH1. Cell Stem Cell 2007; 1(5): 485–487.
16. Xu SL, Zeng DZ, Dong WG, et al. Distinct patterns of ALDH1A1 expression predict metastasis and poor outcome of colorectal carcinoma. Int J Clin Exp Pathol 2014; 7(6): 2976–2986.
17. Lim JS, Kim D, Baek SE, et al. Perfusion MRI for the prediction of treatment response after preoperative chemoradiotherapy in locally advanced rectal cancer. Eur Radiol 2012; 22(8): 1693–1700.

doi: 10.18282/amor.v1.i2.15