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Aldehyde dehydrogenase 1B1 (ALDH1B1) is a potential biomarker for human colon cancer

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ABSTRACT

Aldehyde dehydrogenases (ALDHs) belong to a superfamily of NAD(P)$^+$-dependent enzymes, which catalyze the oxidation of endogenous and exogenous aldehydes to their corresponding acids. Increased expression and/or activity of ALDHs, particularly ALDH1A1, have been reported to occur in human cancers. It is proposed that the metabolic function of ALDH1A1 confers the "stemness" properties to normal and cancer stem cells. Nevertheless, the identity of ALDH isozymes that contribute to the enhanced ALDH activity in specific types of human cancers remains to be elucidated. ALDH1B1 is a mitochondrial ALDH that metabolizes a wide range of aldehyde substrates including acetaldehyde and products of lipid peroxidation (LPO). In this study, we immunohistochemically examined the expression profile of ALDH1A1 and ALDH1B1 in human adenocarcinomas of colon ($N = 40$), lung ($N = 30$), breast ($N = 33$) and ovary ($N = 33$) using an NIH tissue array. The immunohistochemical expression of ALDH1A1 or ALDH1B1 in tumor tissues was scored by their intensity (scale = 1–3) and extensiveness (% of total cancer cells). Herein we report a 5.6-fold higher expression score for ALDH1B1 in cancerous tissues than that for ALDH1A1. Remarkably, 39 out of 40 colonic cancer specimens were positive for ALDH1B1 with a staining intensity of 2.8 ± 0.5. Our study demonstrates that ALDH1B1 is more profoundly expressed in the adenocarcinomas examined in this study relative to ALDH1A1 and that ALDH1B1 is dramatically upregulated in human colonic adenocarcinoma, making it a potential biomarker for human colon cancer.

1. Introduction

The ALDH superfamily of enzymes catalyze the NAD(P)$^+$-dependent oxidation of wide varieties of endogenous and exogenous aldehyde substrates to their corresponding acids [1]. To date, twenty mouse *Aldh* and nineteen human *ALDH* genes have been reported to encode proteins; functional polymorphisms in these genes are associated with distinct phenotypes in humans and rodents [2], indicating that these ALDH enzymes play diverse but physiologically important roles. In recent years, the ALDH activity has been used as a specific marker for the selection of normal and cancer stem cells [2]. It was first found that human hematopoietic stem cells (HSCs) were rich in ALDH activity [3]. Through the use of a synthetic fluorescent substrate for ALDH, named BODIPY aminoacetaldehyde (BAAA), primitive HSCs were effectively isolated from human umbilical cord blood using fluorescence activated cell sorting [3]. Later studies provided several lines of evidence supporting a critical role of ALDH-mediated retinoic acid (RA) signaling in regulating HSC self-renewal and differentiation [4,5]. It was hypothesized that the main ALDH isozyme involved was ALDH1A1 due to its ability to convert retinol to RA [5]. More recently, the functional activity of ALDH has been widely used to identify and isolate cancer stem cells (CSCs) found in the bone marrow [6], breast [7], lung [8], ovary [9], colon [10], prostate [11], and pancreas [12]. In these studies, a commercially available ALDEFLUOR assay was used to sort out a subpopulation of cells possessing high ALDH activity (ALDH^{bri} cells). The ALDH^{bri} cells, in some cases simultaneously positive for other markers of stem cells, revealed much higher tumorigenic potential than ALDH^{low} cells based on *in vivo* tumorigenicity and *ex vivo* clonogenicity assays. Similarly, the expression of ALDH enzymes, mainly ALDH1A1, was found to be upregulated in tumor samples by immunohistochemical studies [9]. The molecular basis of ALDH1A1, and likely other ALDHs, being selectively expressed in CSCs is not completely understood. It is

Abbreviations: ALDH, aldehyde dehydrogenase; LPO, lipid peroxidation; BAAA, BODIPY aminoacetaldehyde; RA, retinoic acid; HSC, hematopoietic stem cell; CSC, cancer stem cell; TARP, Tissue Array Research Program.

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proposed, however, that the metabolic activity of ALDH1A1 towards cytotoxic aldehydes arising from chemotherapies is a key determinant of cell survival and drug resistance of CSCs [8,13]. Nevertheless, since the existing ALDEFLUOR assay selects only for the total ALDH activity, without specificity for individual ALDH isozymes, the identity of specific ALDH isozyme(s) in contributing to the enhanced ALDH activity in a specific type of human cancers remains to be elucidated.

ALDH1B1 is a mitochondrial ALDH isozyme and shares 65% homology in peptide sequence with cytosolic ALDH1A1 in human. We have recently characterized the biochemical properties of ALDH1B1 enzyme using human recombinant ALDH1B1 [14]. Human ALDH1B1 is catalytically active towards a wide range of aldehyde substrates, including aliphatic and aromatic aldehydes and the products of LPO. Furthermore, ALDH1B1 possesses the second lowest k_m (55 μM) among ALDHs for the oxidation of acetaldehyde, supporting its putative role in ethanol metabolism. By immunohistochemical analysis, human ALDH1B1 was found to be expressed at high levels in the small intestine, liver, and pancreas, and at lower levels in the lung and colon [14]. At present, the physiological and/or pharmacological-toxicological role(s) of ALDH1B1 are largely unknown. To begin to understand the role of ALDH1B1 in cancer biology, we examined the expression profile of ALDH1B1 and ALDH1A1 in human adenocarcinomas of the colon, lung, breast and ovary by immunohistochemical staining. Our results demonstrated that the two enzymes are differentially expressed in each tissue examined, and the ALDH1B1 protein appears to be more highly expressed in human epithelial cancers than is ALDH1A1.

2. Materials and methods

2.1. Human tissues

Formalin-fixed and paraffin-embedded tissues of normal human liver, colon, lung, breast and ovary were procured by IHCtech (Aurora, CO) in accordance with relevant approvals and guidelines for use in immunohistochemical staining. Human cancer tissues of the colon, lung, breast and ovary were purchased as tissue microarray slides (T-MTA-6A) from the Cooperative Human Tissue Network and the Tissue Array Research Program (TARP) of the National Cancer Institute (NIH, Bethesda, MD). Patient diagnosis, gender, age and cancer characteristics were provided by TARP.

2.2. Cross-reactivity of ALDH antibodies

The immuno-reactivity of anti-human ALDH1A1 (BD Pharmingen, San Diego, CA) and anti-human ALDH1B1 [14] towards six ALDH isozymes were assessed by Western blotting analysis (see Supplementary material).

2.3. Immunohistochemical staining (IHC)

IHC was performed by IHCtech according to a standard procedure [15] with minor modifications. Briefly, paraffin-embedded tissue sections (5 μm) were deparaffinized and rehydrated, followed by heat-induced epitope retrieval in Retrieval Solution (Leica Microsystems, Bannockburn, IL) at 90 °C for 10 min. After incubation with Protein Blocker (Open Biosystems, Huntsville, AL), sections were incubated with monoclonal anti-human ALDH1A1 (BD Pharmingen, San Diego, CA) at 1:1000 or polyclonal anti-human ALDH1B1 [14] at 1:750 in Protein Blocker for 60 min at room temperature. Corresponding HRP-conjugated secondary antibodies (BioCare Medical, Concord, CA) were used at 1:500 for 10–20 min at room temperature. Slides were incubated with DAB (Open Biosystems, Huntsville, AL) for 10 min, followed by counterstaining with Hematoxylin. Negative control slides were processed in the same manner except that Protein Blocker was applied replacing anti-human ALDH1A1 or pre-immune serum was applied replacing anti-human ALDH1B1.

2.4. Cancer sample analysis

Tissue sections of adenocarcinoma samples of colon, lung, breast and ovary (50 samples each) were present on the tissue microarray slide and each was evaluated for the immunopositivity of ALDH1A1 and ALDH1B1 staining as described [16]. Briefly, the IHC signal for each antibody in each tissue was rated semi-quantitatively in positively stained sections for intensity (I) on a scale of 1–3 (1 = faint staining, 2 = moderate staining, and 3 = strong staining) and for extensiveness (E, % of tumor cells showing an IHC signal). Samples were examined and scored by two independent observers (YC and DJO). Those samples lacking enough tumor tissue for evaluation were excluded from the final analysis. The IHC expression score (S) for each antibody in each sample was calculated by $S = I \times E$. Data are reported as mean ± SEM.

3. Results

3.1. Evaluation of the cross-reactivity of anti-human ALDH1A1 and anti-human ALDH1B1

Before starting our IHC survey of tissues, the specificity of the antibodies was evaluated. The monoclonal anti-human ALDH1A1 is commercially available and has been used in numerous studies for the immuno-detection of ALDH1A1 protein [9,10,17]. The polyclonal anti-human ALDH1B1 antibody was raised against a 58-peptide (amino acids 353–411) of human ALDH1B1 and purified through an antigen-bound affinity column [14]. We examined the immunoreactivity of these antibodies towards six human recombinant ALDH proteins by Western immunoblotting analysis (see Supplementary material). Under the conditions we used, the anti-ALDH1A1 antibody reacted exclusively with ALDH1A1 protein; on the other hand, the anti-ALDH1B1 antibody exhibited strongest immunoreactivity towards ALDH1B1 protein, albeit showing some degree of cross-reactivity to ALDH1A1 protein and no cross-reactivity to other ALDH proteins.

3.2. ALDH1A1 and ALDH1B1 distribution in normal human tissues

IHC staining was performed in normal human tissues of liver, colon, lung, breast and ovary for the presence of ALDH1A1 and ALDH1B1 protein, respectively (Fig. 1).

3.2.1. ALDH1A1 expression (Fig. 1A)

In the liver, both hepatocytes (*boxed area*) and cholangiocytes (*arrow*) showed immunopositivity, whereas most stromal cells were negative. The staining of hepatocytes was of a "checkerboard pattern", showing high variability from cell to cell without apparent order. Hepatocytes in the peri-portal and centrilobular portions exhibited similar quantities of ALDH1A1 staining, which showed both a cytoplasmic and a plasma membrane component. The cholangiocyte staining was more uniform and only appeared to be of the cytoplasmic variety. In the colon, we observed a cytoplasmic staining of ALDH1A1 both in cells located at the bottom of the crypts (*arrows*) as well as in many of the partially differentiated absorptive and secretory cells at higher crypt levels (*arrowheads*), whereas the fully differentiated cells were negative. A few of the stromal macrophages were slightly positive, while other stromal cells were negative. In the lung, the Clara cells showed strong cytoplasmic immunopositivity; the basal cells and ciliated cells were also positive, but to a lesser extent. Stromal elements appeared negative. In the breast, ALDH1A1 immunopositivity was sparse and included only some of the luminal epithelial cells (*arrows*);