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Acetaldehyde and Retinaldehyde-Metabolizing Enzymes in Colon and Pancreatic cancers

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Abstract

Colorectal (CRC) and pancreatic cancers are two very significant contributors to cancer-related deaths. Chronic alcohol consumption is an important risk factor for these cancers. Ethanol is oxidized primarily by alcohol dehydrogenases to acetaldehyde, an agent capable of initiating tumors by forming adducts with proteins and DNA. Acetaldehyde is metabolized by ALDH2, ALDH1B1 and ALDH1A1 to acetate. Retinoic acid (RA) is required for cellular differentiation and is known to arrest tumor development. RA is synthesized from retinaldehyde by the retinaldehyde dehydrogenases, specifically ALDH1A1, ALDH1A2, ALDH1A3 and ALDH8A1. By eliminating acetaldehyde and generating RA, ALDHs can play a crucial regulatory role in the initiation and progression of cancers. ALDH1 catalytic activity has been used as a biomarker to identify and isolate normal and cancer stem cells; its presence in a tumor is associated with poor prognosis in colon and pancreatic cancer. In summary, these ALDHs are not only biomarkers for CRC and pancreatic cancer but also play important mechanistic role in cancer initiation, progression and eventual prognosis.

Keywords

Acetaldehyde; ALDH; Biomarker; Colorectal cancer; Pancreatic cancer; Retinaldehyde; Stem cells

Introduction

Colorectal cancer (CRC) and pancreatic cancer represent serious health concerns because of their very high morbidity and mortality. Each year, more than one million new CRC cases are diagnosed and over 500,000 deaths are associated with this condition worldwide [1]. In the USA, CRC is the fourth most commonly diagnosed cancer and second leading cause of cancer-related death. Pancreatic cancer ranks tenth in incidence but is disproportionately

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fatal in being the fourth largest cause of cancer-related deaths in the USA. The American Cancer Society estimated diagnosis of 142,820 new cases of CRC in the USA during 2013; of these, approximately 50,830 people are expected to die. The estimated incidence of pancreatic cancer is 45,220 with 38,460 deaths [2]. Although the exact mechanisms that promote CRC remain obscure, there is increasing evidence suggesting the involvement of lifestyle-related factors in addition to genetic predisposition. These factors include waist circumference, folate and multivitamins in the diet, high fat and high energy diet, physical exercise, tobacco smoking and alcohol consumption [3, 4].

According to dose-response meta-analysis and pooled results from cohort studies, chronic daily consumption of approximately 50 g alcohol increases the relative risk for colon cancer by 40 per cent [5, 6]. Alcohol and its primary metabolite, acetaldehyde, have also been linked with pancreatic cancer [7]. Various theories have been advanced regarding the mechanism by which alcohol induces cancer. For example, ethanol may enhance mucosal penetration of a carcinogen by serving as a solvent. In addition, ethanol induces cytochrome P4502E1 (CYP2E1), an enzyme capable of generating reactive oxygen species (Fig. 1). However, the most well accepted theory regarding ethanol-induced cancer involves acetaldehyde acting as a carcinogen [8, 9]. Ethanol is metabolized to acetaldehyde by alcohol dehydrogenase (ADH), CYP2E1 and catalase [10, 11] (Fig. 1). Acetaldehyde is a molecule capable of forming adducts with DNA which is considered an initial step in carcinogenesis [12]. In 2009, the International Agency for Research on Cancer (IARC) designated acetaldehyde (as associated with alcohol consumption) to be a group I human carcinogen [13]. Acetaldehyde is metabolized to acetate, a process catalyzed by aldehyde dehydrogenase (ALDH) 2, ALDH1B1 and ALDH1A1 (Fig. 1) [14]. The ability of these ALDHs to repress cellular acetaldehyde levels is consistent with a role for ALDHs in colon and pancreatic cancers and is supported strongly by the association of ALDH2 deficiency with high incidence of CRC and pancreatic cancer in heavy ethanol drinkers [15, 16]. In addition to metabolizing acetaldehyde, ALDH1 isozymes are the primary enzymes involved in the metabolism of retinaldehyde to retinoic acid (RA), a signaling molecule that plays a crucial role in cellular proliferation and differentiation [11]. Given their ability to affect cellular RA levels, it is likely that RA-generating ALDHs have a role in modulating carcinogenesis. Several other observations lend support to the notion that ALDHs are implicated in cancer. First, ALDH activity has been used to identify and isolate normal and cancer stem cells of various lineages [17–19] (Table 1). Second, high ALDH expression has been found to be associated with poor clinical outcome in leukemia [20], ovarian [21–23], prostate [24, 25], breast [26–28], colorectal [29] and pancreatic cancer [15, 30]. Third, ALDH+ cells (cells with very high ALDH expression) exhibit a greater tumorigenic capacity, as reflected in colony-forming capability *in vitro* and in xenograft-induced tumor formation *in vivo* [31]. We have found very strong up-regulation of ALDH1B1 expression in an animal model of colon polyps, specifically adenomatous polyposis coli multiple intestinal neoplasia (*Apc*(*Min*)/+) mice (our unpublished data). These mice have point mutation in *Apc*, a tumor suppressor gene which when mutated leads to dysregulation of the Wnt-signaling pathway and results in up-regulation of oncogenes like *c-Myc* [32]. Overexpression of ALDH1B1 in polyps from these mice is suggestive of a possible

relationship between Wnt-signaling and ALDH1B1 expression, a consideration that warrants further study.

A causal relationship exists between alcohol consumption and CRC or pancreatic cancer and this may be mediated, at least in part, by acetaldehyde [12, 15]. The significance of retinaldehyde and acetaldehyde in tumor formation, and very high expression of the ALDHs in colorectal and pancreatic cancer are suggestive of a crucial role for acetaldehyde-and retinaldehyde-metabolizing ALDHs in these cancers. Lack of ALDH2 activity and resultant high acetaldehyde levels are linked with colon cancer initiation. By contrast high ALDH1 activity (primarily ALDH1A1 and ALDH1B1) is required for the stemness and tumorigenic potential of cancer stem cells.

Acetaldehyde: A carcinogen

Acetaldehyde is categorized as 'carcinogenic to humans' and 'reasonably anticipated to be a human carcinogen' according to IARC regulations and United States National Toxicology Program (NTP), respectively [13, 33]. Acetaldehyde has been shown to be a highly toxic, mutagenic and carcinogenic compound in a variety of *in vitro* and *in vivo* studies. Its effects range from damaging antioxidant defenses [11] to interfering with DNA methylation and repair mechanisms through formation of adducts with DNA and proteins (Fig. 1) [10, 12]. In the colon, acetaldehyde is primarily produced from ethanol by resident bacteria and, to a lesser extent, by mucosal ADHs. As a result of metabolism by intra-colonic microbes, large quantities (nine-fold higher than normal) of acetaldehyde accumulate in the rat colon 2 hrs after intra-peritoneal injection of ethanol [34]. Human colon mucosal cells harbor ADH1, ADH3 and ADH5, with the ADH1 and ADH3 isozymes being most active [35]. In an *in vitro* experiment, human colon contents were able to generate 60 to 250 μM acetaldehyde when incubated with concentration of ethanol (10–100 mg%), which is known to be attained during normal ethanol drinking [36]. The high levels of acetaldehyde attained in the colon after drinking ethanol likely underlies the correlation between chronic, heavy ethanol consumption and CRC in humans. In ethanol-treated rats, a high concentration of acetaldehyde (50 to 350 μM) in the colon mucosa has been shown to correlate positively with hyper-proliferation of the colon crypt cells. Such a phenomenon would be anticipated to favor the development of CRC [37, 38].

Acetaldehyde is metabolized primarily by mitochondrial ALDH2 and ALDH1B1 and, to lesser extent, by cytosolic ALDH1A1 (Table 2) [14]. The most convincing evidence for a role of acetaldehyde in CRC initiation emanates from studies involving Asians who possess a polymorphism in their ALDH2 enzyme known as ALDH2*2. These subjects possess a single nucleotide polymorphism (SNP) that leads to a lysine to glutamate substitution at residue 504 that renders the enzyme functionally-inactive [39]. Approximately 40 per cent of the Asian population carry an ALDH2*2 allele; this compromises their ability to metabolize acetaldehyde and increases their colon cancer risk 3.4 times [16].

Opposing effects of retinoic acid on cancer cell proliferation

Retinoids exert many physiologically-important and diverse functions in relation to cellular proliferation and differentiation of normal and cancer cells. For example, retinoids are

crucial for embryonic development and adult tissue remodeling. The retinoids comprise all of the derivatives of retinol, including all-*trans*-, 9-*cis*- and 13-*cis*-retinoic acid (RA). Retinol is oxidized to retinaldehyde by retinol dehydrogenases. The resultant retinaldehydes are further metabolized to their corresponding RA by retinaldehyde dehydrogenases which include RALDH1 (ALDH1A1), RALDH2 (ALDH1A2), RALDH3 (ALDH1A3) and RALDH4 (ALDH8A1) (Table 2) [40–45]. Among the RAs, all-*trans*-RA (ATRA) is the most biologically potent retinoid. Abnormally low levels of ALDH1A2 have been observed in breast and prostate cancers [46, 47]. Impaired RA formation and high levels of CYP26A1 (a RA-metabolizing enzyme) in human breast cancer are consistent with a protective role for RA in this cancer [46–48]. The physiological actions of the retinoids are mediated through binding of the RA receptor (RAR) and retinoid X receptor (RXR) heterodimer to the regulatory region of retinoid-responsive genes, known as RA response elements [49]. RARs and RXRs are ligand-dependent transcription factors and exist as α, β or γ isoforms. RAR isoforms interact with both ATRA and 9-*cis* RA, whereas RXR isoforms interacts only with 9-*cis* RA [50, 51]. The binding of RA with the RAR/RXR dimer recruits co-activator proteins and initiates transcriptional activation of the retinoid-responsive genes [49]. Retinoids have been found to be effective for the treatment of acute promyelocytic leukemia and prevention of liver, lung, breast, prostate, skin and colon cancers [52–54]. *In vivo* studies involving rats have revealed that retinoids added to the diet reduced colon cancer cell proliferation and prevented azoxymethane-induced aberrant crypt foci (putative precancerous lesions in colon) and colon tumor formation [54, 55]. A RXR-selective retinoid, AGN194204, has been found to inhibit the proliferation of human pancreatic cancer cells, an effect that can be reversed by a RXR-selective antagonist [56]. In addition to inhibiting the growth of pancreatic cancer cells, RA increases the sensitivity of pancreatic adenocarcinoma cells to the antineoplastic drugs gemcitabine and cisplatin [57].

In contrast to the anti-proliferative and anti-survival role of RA in cancer cells, dietary ATRA has been shown to enhance initiation and growth of intestinal tumors in the *Apc (Min)*/+ mouse model *in vivo* [58]. RA can promote cell survival and hyperplasia in cells expressing high levels of fatty acid-binding protein 5 (FABP5) by activating an orphan nuclear receptor, peroxisome proliferator-activated receptor (PPAR)β/δ [59]. PPARβ/δ mediates antiapoptotic properties partly by inducing the PDK1/Akt survival pathway [60]. RA binds to intracellular lipid binding proteins (iLBPs), including cellular retinoic acid-binding proteins (CARBPII) and FABP5. CARBPII and FABP5 are selective for nuclear receptors RARα and PPARβ/δ, respectively [59]. Hence, RA induces CARBPII- or FABP5-mediated activation of RAR or PPARβ/δ (respectively), depending on the ratio of FABP5/CRBPII in the cells [59]. Human colorectal cancer cell lines (specifically, T84, COLO205, SW620, SW480, HCT116 and DLD-1) express ~30-fold higher levels of FABP5 relative to normal colorectal cells (CCD18-Co), suggesting the possibility of pro-proliferative and anti-apoptotic roles for RA in these cells [59, 61]. However, the expression levels of PPARβ/δ in colorectal cancer cells and its role in tumorigenesis are unresolved in various cancers, including CRC [62].

RA inhibits the proliferation and increases chemosensitivity of pancreatic cancer cells. However, the involvement of RA in CRC is less clear, with opposing findings suggesting pro- or anti-proliferative roles.

ALDH and cancer stem cells

In the gastrointestinal (GI) tract, tissue-specific stem cells are at the top of the cellular hierarchy and play a critical role in regulating tissue homeostasis. These specialized epithelial cells are characterized by their ability to self-renew and differentiate into a variety of cellular populations that perform specific functions within the GI tract. Currently, it is believed that these tissue-specific stem cells (or progenitor cells), when oncogenically transformed, become cancer stem cells (CSCs) or tumor initiating-cells (TICs) since they functionally possess the capacity to form tumors and maintain tumor growth. Accumulating evidence also suggests that CSCs are responsible for chemotherapeutic/radiation resistance and tumor recurrence (Fig. 2). ALDH catalytic activity has been identified in many human cancers [28] and, as such, is used as a marker of CSCs, including colorectal and pancreatic cancer. The pathophysiological function of ALDH in CSCs remains unresolved. Intense research of ALDH enzymes is underway in order to elucidate the role of these proteins in the development and progression of cancer as well as drug resistance.

Colorectal cancer

Although earlier stages of CRC are highly curable, therapeutic interventions in advanced disease have proven to be poorly effective at increasing the 5-year survival rate. Recent drug development has focused on targeting the CSC population as a potential therapy. In normal colon, CSC's reside at the bottom of the crypt and generate upward, migrating and differentiating transit amplifying cells (in the middle of the crypt) which become terminally differentiated cells as they move upward and eventually shed into the lumen (Fig. 3A) [63]. In CRC, several different molecules, including the cell surface markers CD133 and CD44 as well as ALDH activity, have been proposed as biomarkers for identification and isolation of the CSC population [31, 64–67]. CD133+ colon cancer cells were initially shown to be tumorigenic [66, 67]. However, subsequent studies identified that both CD133+ and CD133- cells possess tumorigenic potential [68]. CD44+ (either with or without epithelial-specific antigen (ESA+)) was demonstrated to be a marker in colon CSCs [68]. However, additional studies showed that CD44+ cells reside throughout the entire crypt, including the proliferative compartment, suggesting that the CD44+ colon cells are not necessarily stem-like [64]. We have examined CD44 and ALDH together in one of our CRC patient-derived tumor xenograft (PDTX) models to determine if CD44+ cells had tumorigenic properties [69]. Despite ALDH+/CD44+ cells showing some tumorigenic growth, ALDH+/CD44- cells exhibited a higher incidence and faster growing tumors. In this same PDTX model, isolation and injection of ALDH+ and ALDH- cells in mice showed a significant difference with respect to tumor growth [69]. ALDH+ cells produced fast growing and large tumors when compared to ALDH- cells that either produced very small tumors or no tumors in five separate PDTX models. Importantly, all ALDH+ tumors looked morphologically the same as the original tumor. Several other studies have shown that injection of ALDH+ cells from colitis and colon cancer patients facilitated spheroid formation (*in vitro* 3-dimensional

spheroid cell culture that more closely resembles the *in vivo* environment) and tumor growth in a xenograft model, while ALDH-cells were incapable of tumor growth [31, 64]. These studies demonstrate that ALDH catalytic activity appears to be a robust marker of CSCs in CRC.

Given the apparent promise of ALDH activity as a potential biomarker of CSCs, many investigations are currently exploring the role of ALDH in CSC function. In particular, a great deal of focus is being placed on which ALDH isoform(s) mediate the catalytic activity in the CSCs. In normal colon stem cells, ALDH1 has been demonstrated to be primarily expressed at the bottom of the crypt compartment in the colon (where colon-specific stem cells are located) and ALDH1 levels are significantly elevated in the development and progression of CRC [64]. Interestingly, ALDH1 protein levels are elevated in the colon of patients with ulcerative colitis (a risk factor for colon cancer) compared to normal colon cells; such expression may be important in the transformation from colitis to colon cancer [31]. We have shown that ALDH1B1 protein is 5.6-fold higher when compared to ALDH1A1 in CRC patients and may be a potential biomarker in CRC (Fig. 3B–C) [70]. Similarly, very high expression of ALDH1B1 was found in the colon polyps of *Apc* (*Min*)/+ mice (our unpublished data). While these studies indicate elevations in individual ALDH isoforms in CRC, the contribution of these enzymes to the progression of CSCs and CRC remain to be clarified.

A common problem associated with standard chemotherapeutic regimens in CRC is treatment resistance. Although chemotherapy is effective at reducing tumor burden, many CRC patients will experience disease recurrence and ultimately succumb to their disease. CSCs are thought to be responsible for chemotherapy-resistance and disease recurrence [71]. Therefore, therapeutic elimination of this population would be predicted to reduce tumor recurrence and ultimately improve survival. In our CRC PDTX model, the effects of an inhibitor of the Notch pathway (considered to be important for self-renewal of colon stem cells) in combination with irinotecan was investigated on the ALDH+ cell population [69]. The combination therapy was effective at reducing the number of ALDH+ cells as well as tumor recurrence, even after treatment was discontinued when compared to single agent Notch pathway inhibition and irinotecan. Administration of the combination therapy for 28 days prevented tumor growth in the ALDH+ cell xenograft model; this protection continued for 3 months after combination treatment was completed [69]. These data indicate that the ALDH+ population has the ability to self-renew, and significantly reducing this population of cells delays tumor recurrence (Fig. 2). Whether specific ALDH isozymes contribute to chemotherapy resistance remains to be determined.

Pancreatic cancer

Despite considerable research, the 5-year survival rate for pancreatic cancer still remains extremely poor. A concerted effort is underway to delineate pathways that are dysregulated in the CSC population of this disease and thereby identify novel potential therapeutic targets.

In pancreatic cancer, ALDH+ cells have been shown to possess stem cell features, as evidenced by enhanced clonogenicity *in vitro* and tumorigenic growth in mice [72]. These cells also have greater tumorigenic potential than CD133+ cells [72]. Interestingly, ALDH+ cells from pancreatic cancers have been demonstrated to: i) express many genes of the mesenchymal phenotype, ii) have an increased capacity to migrate and invade, and iii) be more numerous in metastatic lesions [30]. Furthermore, in pancreatic cancer patients, expression of ALDH in tumors is associated with a worse survival rate than those tumors that do not express ALDH [30]. ALDH1A1 expression has been linked to resistance to chemotherapy in a pancreas PDTX model. In this context, treatment with gemcitabine was shown to enhance gene and protein expression of ALDH. Inclusion of an inhibitor of hedgehog (a pathway important for stem cell regulation in the pancreas) with gemcitabine resulted in decreased expression of ALDH [73]. These studies suggest that ALDH+ cells are stem-like cells in pancreas cancer and may be important contributors in disease progression and chemoresistance; therefore contribute to the negative outcomes in patients with pancreatic cancer.

Summary

There is accumulating evidence that supports a role for ALDHs in cancer development and progression. The exact mechanisms by which ALDHs influence tumorigenesis remain to be defined. Certainly, metabolism of acetaldehyde and/or the generation of retinoic acid represent modalities by which ALDHs could influence CRC and pancreatic cancer. ALDH catalytic activity appears to be an excellent biomarker that can be utilized for the isolation and characterization of the CSC population in tumors obtained from patients with CRC or pancreatic cancer. It is becoming apparent that the various ALDH isozymes may have different roles in tumorigenesis (from metabolism of the carcinogen to modulation of the proliferation-regulating retinoids) and that the timing and cellular localization of isozyme expression may be critical factors that influence how ALDHs modulate cancer development and progression. Further studies are needed that identify (i) the importance of ALDH catalytic activity in modulation of tumorigenesis, (ii) the specific ALDH isozymes involved (and that regulate CSCs), and (iii) the signaling pathways that regulate tumor-associated ALDH expression. The results obtained from such studies should lead to the development of novel therapies that may more effectively treat these devastating diseases.

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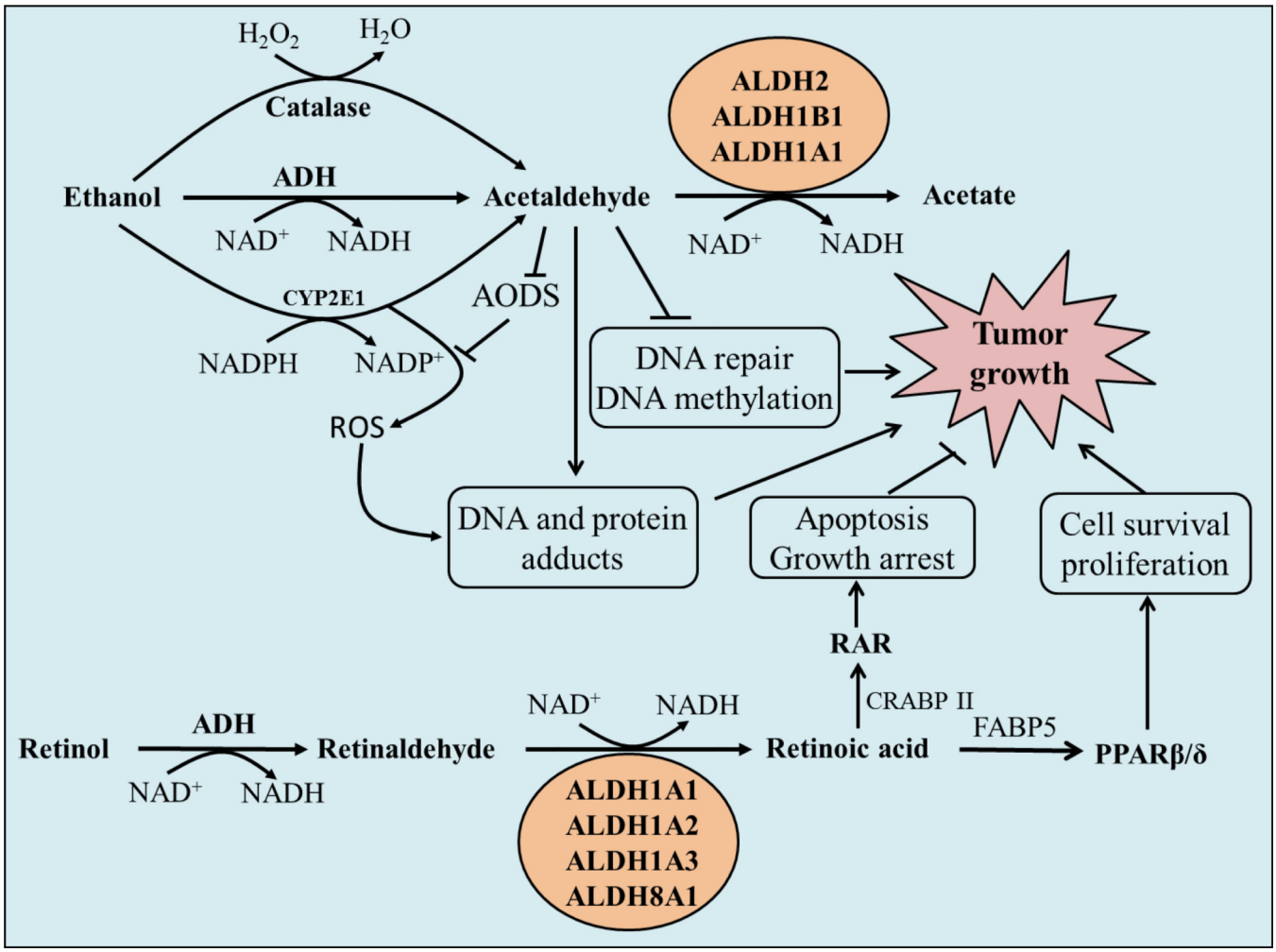

Fig. 1. ALDHs modulate carcinogenesis by metabolizing acetaldehyde and retinaldehyde

Ethanol is metabolized by alcohol dehydrogenase (ADH), catalase and CYP2E1 to acetaldehyde. Acetaldehyde can interfere with anti-oxidative defense systems (AODS) and generate reactive oxygen species (ROS); inhibits DNA repair and methylation; and forms DNA and protein adducts to promote tumor growth. Acetaldehyde is metabolized to acetate primarily by ALDH2, ALDH1B1 and ALDH1A1. Retinaldehyde, formed from retinol by ADH, is converted to retinoic acid (RA) by retinaldehyde-metabolizing ALDHs. RA exerts anti-carcinogenic activity by binding to cellular retinoic acid binding proteins (CRBPII) and activating the RA receptor (RAR). When RA binds to fatty acid binding protein 5 (FABP5), it activates orphan nuclear receptor peroxisome proliferator-activated receptor (PPAR)β/δ and acts as procarcinogenic agent. ALDH, aldehyde dehydrogenase; NAD^+, NAD(P), nicotinamide adenine dinucleotide (phosphate); H_2O_2, hydrogen peroxide.

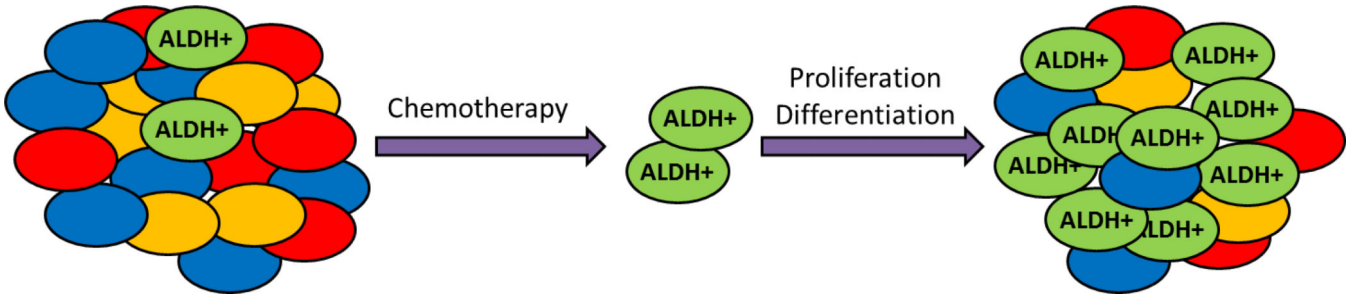

Fig. 2. ALDH-expressing cells are responsible for chemoresistance and relapse of many tumors after chemotherapy

Most current chemotherapy drugs are effective against the bulk of the tumor cells. However, the high ALDH-expressing (ALDH+) cancer stem cells are resistant to these treatments. As a result, during chemotherapy, the ALDH+ cells proliferate and promote tumor growth. The resultant tumors contain an increased proportion of ALDH+ cells, making them more resistant to chemotherapy than the original tumor.

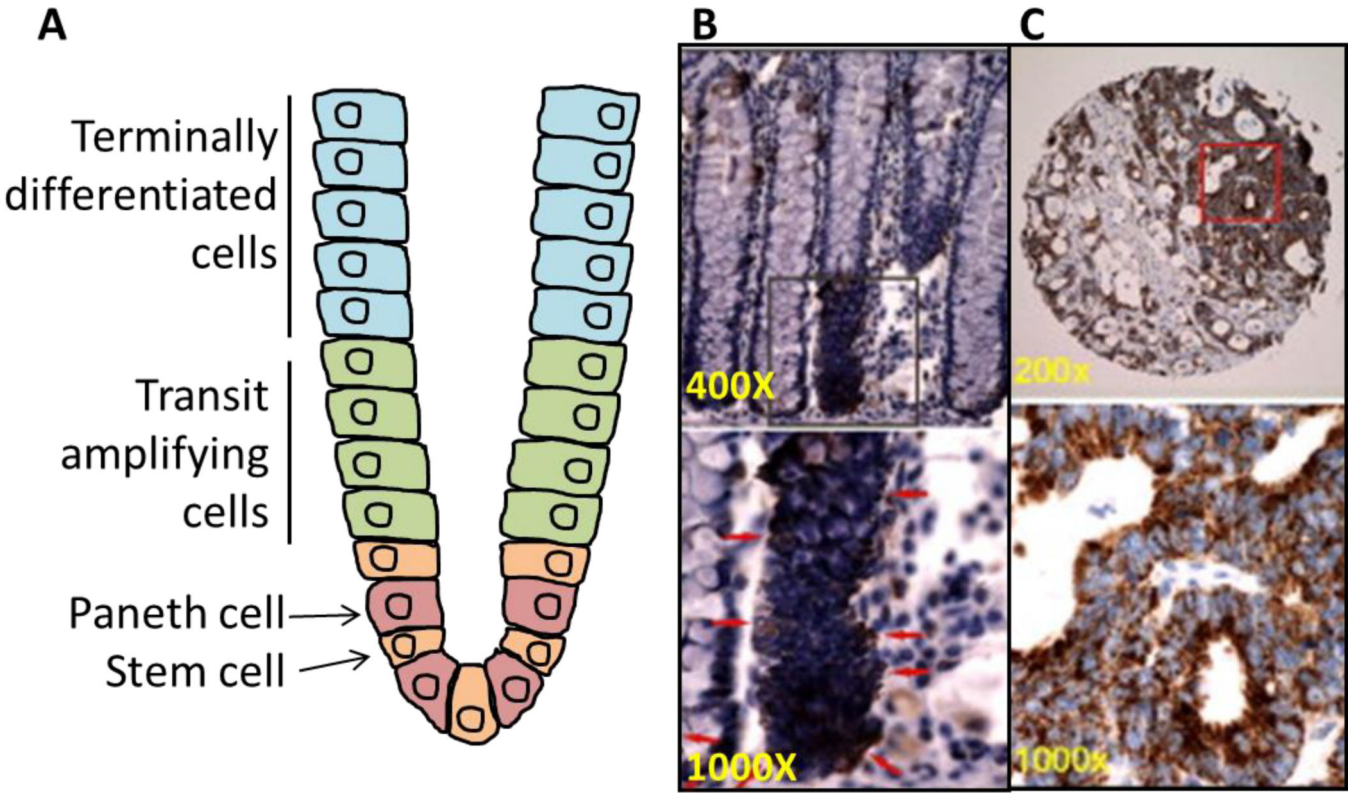

Fig. 3. ALDH1B1 expression pattern in normal colon and colon adenocarcinoma

Location of various cell types in normal colon (**A**). ALDH1B1 expression (red arrows) is strictly localized to stem-like cells at the base of crypts in the normal human colon (**B**). ALDH1B1 is expressed at extremely high levels throughout all cells of human colon adenocarcinomas (**C**). In figures B and C (reproduced from Chen et al., 2011 [70]), lower panels are higher magnification of areas identified by squares in the upper panel.

Table 1

ALDH expression in various progenitor, stem and cancer cell types.

S.No.	Cell or tumor type	ALDH isozyme(s) [a]	Reference
1	Hematopoietic progenitor	ALDH, ALDH1A3	[17–19, 74, 75]
2	Mesenchymal progenitors	ALDH	[74]
3	Endothelial progenitors	ALDH	[74]
4	Neural stem cells	ALDH, ALDH1L1	[76, 77]
5	Normal mammary stem cells	ALDH1A1	[26]
6	Breast cancer stem cells	ALDH1A1, ALDH1A3, ALDH2, ALDH6A1,	[26, 28, 75, 78, 79]
7	Prostate cancer	ALDH, ALDH7A1	[24, 25, 80]
8	Ovarian cancer stem cells	ALDH, ALDH1A1	[21–23, 81]
9	Ovarian cancer cells	ALDH1A1, ALDH1A3, ALDH3A2, ALDH7A1	[82]
10	Colon stem cells	ALDH1A1, ALDH1B1	[64, 70]
11	Colon cancer stem cells	ALDH1A1, ALDH1B1	[21, 29, 31, 64, 70, 83]
12	Leukemia stem cells	ALDH	[20]
13	Human lung cancer cells	ALDH1A1	[21, 84, 85]
14	Head and neck cancer stem cells	ALDH1A1	[86]
15	Pancreatic cancer	ALDH, ALDH1A1, ALDH1A3	[30, 75, 87]
16	Liver cancer stem cells	ALDH, ALDH1A1	[88, 89]

[a] ALDH is designated for studies in which ALDH+ cells were identified and isolated using the ALDEFLUOR™ assay.

Table 2

Affinity of ALDHs for acetaldehyde and retinaldehyde

S.No.	ALDH isozyme(s)	Substrate	K_m	Reference
1	ALDH1A1	Acetaldehyde	180 μM	[14]
		All-*trans* Retinaldehyde 9-*cis* Retinaldehyde	11.6–26.8 μM 3.59 μM	[90], Jackson et al, under preparation.
2	ALDH1A2	All-*trans* Retinaldehyde 9-*cis* Retinaldehyde	0.66 μM 0.62 μM	[91]
3	ALDH1A3	All-*trans* Retinaldehyde	0.2 μM	[92]
4	ALDH1B1	Acetaldehyde	55 μM	[14]
		Retinaldehyde	24.9 μM	Jackson et al., under preparation
5	ALDH2	Acetaldehyde	3.2 μM	[14]
6	ALDH8A1	9-*cis* Retinaldehyde	3.15 μM	[45]