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TISSUE-DISTRIBUTION OF ALDEHYDE DEHYDROGENASE 2 AND EFFECTS OF THE ALDH2 GENE-DISRUPTION ON THE EXPRESSION OF ENZYMES INVOLVED IN ALCOHOL METABOLISM

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1. ABSTRACT

In alcohol metabolism, acetaldehyde, a highly reactive intermediate that may cause cellular and DNA damages, is converted to acetate by mitochondrial aldehyde dehydrogenase ALDH2. Although the majority of ingested alcohol is eliminated in the liver, the first-pass metabolism of ethanol in the upper digestive tract is also important for prevention and management of ethanol-related gastrointestinal diseases. However, the tissue-distribution of Aldh2 in mice has been poorly investigated. In this study, therefore, we investigated the tissue-distribution of Aldh2 as well as Aldh1, Cyp1a1, Cyp2e1, and Cyp4b1 in wild type and Aldh2-null mice by immuno-histochemical analysis. The human liver and esophageal tissues were also examined. In mice, the Aldh2 protein was detected in the liver, lung, heart, kidney, testis, esophagus, stomach, colon, and pancreas, suggesting that the tissue-distribution of Aldh2 in mice is similar to that in humans. Therefore, Aldh2-null mice may be useful model animals for the investigation of alcohol metabolism and related diseases. Compared with the wild type, the expression level of Cyp2e1 was increased in the liver from Aldh2-null mice based on Western blot analysis, whereas the levels of Aldh1, Cyp1a1, and Cyp4b1 were indistinguishable. This observation suggests that a metabolite(s) of Aldh2 might down-regulate the expression of Cyp2e1 gene.

2. INTRODUCTION

Alcohol misuse is linked to a wide variety of social and medical problems. Direct costs for medical problems related to alcohol abuse in Japan are estimated to have been 1174 billion yen during 1987 (1). Number of Japanese alcoholism patients was about 2.5 million in 1995 and has gradually increased (1). Alcohol misuse causes harmful consequences for many organs, which is associated with the incidence of various cancers, such as esophageal cancer. Therefore, the metabolic pathway of ethanol and its variation among individuals are of great interest for the risk assessment and prevention of diseases caused by alcohol abuse.

In humans, more than 90% of ingested alcohol is eliminated via metabolic degradation mainly in the liver. Ethanol is first converted to acetaldehyde by alcohol dehydrogenase (ADH) although cytochrome P4502E1 (CYP2E1) and catalase also contribute the aldehyde formation. Acetaldehyde is subsequently metabolized into acetate by aldehyde dehydrogenase (ALDH) that requires NAD^+ as a cofactor (2). In humans, there are multiple forms of ALDH that consist of nine major families, ALDH1 to ALDH9. These ALDH enzymes are divided into two groups; cytoplasmic forms (ALDH1, ALDH3, ALDH7, ALDH8, and ALDH9) and mitochondrial forms (ALDH2, ALDH4, ALDH5 and ALDH6) (3, 4). ALDH1A1, ALDH1B1 and ALDH2 play a significant role in acetaldehyde oxidation while the others metabolize a variety of substrates (5). Of the three isoforms involved in acetaldehyde metabolism, the mitochondrial ALDH2 plays a major role in human acetaldehyde metabolism because of its low Km (< 5 μM) for acetaldehyde.

ALDH2*2, a genetic polymorphism of ALDH2, having an amino acid substitution from glutamic acid at 487 to lysine (E487K) is widely prevalent in some Asian

Table 1. Antibodies and conditions for Aldh and Cyp immunohistochemistry

Antigen	Source of primary antibody	Primary antibody	Secondary antibody	Blocking	Dilution (folds)
Aldh1	Dr. Weiner [1]	Rabbit anti-human	Goat anti-rabbit polyclonal antibody	Goat serum	200
Aldh2	Dr. Weiner [1]	Rabbit anti-human	Goat anti-rabbit polyclonal antibody	Goat serum	200
Aldh2	This laboratory [2]	Rabbit anti-mouse	Goat anti-rabbit polyclonal antibody	Goat serum	2000
Cyp1a1	Commercial antibody [3]	Goat anti-rat	Rabbit anti-goat polyclonal antibody	Rabbit Serum	200
Cyp2e1	Commercial antibody [3]	Goat anti-rat	Rabbit anti-goat polyclonal antibody	Rabbit serum	200
Cyp4b1	Commercial antibody [3]	Rabbit anti-mouse	Goat anti-rabbit polyclonal antibody	Goat serum	200

[1]: Supplied by Dr. Weiner, University Of Purdue (29), [2]: Created In Our Laboratory, UOEH (3) [3] Daiichi Pure Chemicals Co., Ltd.

populations (6). Because ALDH2 functions as a homotetramer, the inactive subunit produced by the ALDH2*2 allele acts in a dominant negative fashion. Therefore, individuals carrying the ALDH2*2 allele show high blood acetaldehyde concentrations after the intake of only moderate alcohol amount (7). In consequence of the decreased acetaldehyde metabolism, the ALDH2*2 allele is associated with alcohol-induced flushing (8), and is also positively related with liver disease, oral cancer and esophageal cancer (9-11) while it negatively effects on coronary heart disease (12). The mice (C57BL/6) lacking Aldh2 (Aldh2 knock-out mice; Aldh2 KO mice) (3) should be useful model animals to investigate the effects of ALDH2 deficiency. However, the tissue-distribution of Aldh2 in mice has not been well investigated. In this study, therefore, we systematically investigate the tissue-distribution of Aldh2 as well as Aldh1 and Cyp enzymes in wild type and the expression levels of the enzymes are compared with those in Aldh2 KO mice to investigate the effects of Aldh2-deficiency on the expression of the enzymes involved in alcohol metabolism. Because the expression of ALDH1 and ALDH2 has remained unknown in the human esophagus, we also examined the expressions of ALDH and CYPs in the human tumor tissues.

3. MATERIALS AND METHODS

3.1. Wild type and Aldh2 KO mice

Male C57BL/6 mice (wild mice), 6 – 10 weeks of age, were purchased from Charles River Japan, Inc. (Yokohama). Male Aldh2 KO mice, 6 – 10 weeks of age, were generated as previously described (3). These mice were housed in specific pathogen-free units of the Division of Animal Care and the protocols for animal use were approved by the Ethics Committee for Animal Use of University of Occupational and Environmental Health. More than five mice were used in each experiment.

3.2. Human liver and esophagus

We examined specimens from one patient with metastatic liver tumor from colon cancer and 5 patients with esophageal cancer who underwent surgical resection at the Department of Surgery II, School of Medicine, University of Occupational and Environmental Health in Kitakyushu, Japan between 1997 and 1999. The protocols for this investigation were approved by the Medical Ethics Committee of University of Occupational and Environmental Health.

3.3. Immunohistochemical Staining

Immunohistochemical staining for the detection of Aldh1, Aldh2, Cyp1a1, Cyp2e1 and Cyp4b1 was performed using organs from at least 5 wild mice and 5 Aldh2 KO mice. From each mouse, 9 organs (lung, heart, kidney, testis, liver, esophagus, stomach, colon and pancreas) were dissected. Mice organs were stained using the avidin-biotin complex (ABC) method (13). Antibodies and conditions were described in Table 1. Briefly, 3 μm sections of each mouse organ were incubated first with a 1:200 or a 1:2000 dilution of primary antibodies against Aldh1, Aldh 2, Cyp1a1, Cyp2e1 or Cyp4b1 for 40 minutes at room temperature, followed by 10 minutes incubation with secondary antibodies and peroxidase-labeled streptavidin. Staining was completed after 5 - 15 minutes incubation with a freshly prepared substrate-chromogen solution.

The expression of ALDH1, ALDH2, CYP1A1 and CYP2E1 was also analyzed in the human specimens of metastatic liver cancer and esophageal epithelium by immunohistochemical detection. Tissue sections (3 μm) of the human specimens were stained using the avidin-biotin complex (ABC) method with peroxidase-labeled streptavidin-biotin antibody (LSAB) kit (13). Antibodies and conditions were described in Table 2. The tissue sections were incubated with a 1:100 or a 1:200 dilution of primary antibodies against ALDH1, ALDH 2, CYP1A1 or CYP2E1 for 40 minutes at room temperature, followed by 10 minutes incubation with secondary antibodies and peroxidase-labeled streptavidin. Staining was completed after 15 minutes incubation with a freshly prepared substrate-chromogen solution.

We separated staining levels of organs into five groups, according to rate of positive staining area. We judged as negative (-) when there was no staining positive area, negative-positive (±) when the rate of positive staining area was less than 5%, positive (+) when the rate was from 5% to 10%, double positive (++) when the rate was from 10% to 50%, triple positive (+++) when the rate was more than 50%.

3.4. Western blot analysis

Cytosolic, mitochondrial and microsomal fractions from mouse livers were prepared as described (14). The protein concentration was estimated by Bradford method (Bio-Rad) (15). Cytosolic proteins were used for the detection of Aldh1, mitochondrial proteins were for Aldh2, and microsomes for Cyp1a1, Cyp2e1 and Cyp4b1. Proteins (20μg each) were separated by 10% SDS-PAGE and transferred onto a polyvinylidene Hybond-P membrane (Amersham, Arlington Heights, IL). Western blot analysis was performed using the indicated antibodies and

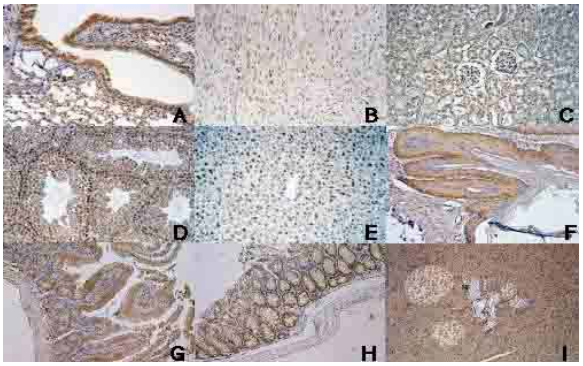

Figure 1. Localization of Aldh1 in mice. The expression of Aldh1 in nine organs from mice were analyzed by immunohistochemistry as described in "Materials and Methods". The alphabet from A to I indicated organs as follows: A, lung; B, heart; C, kidney; D, testis; E, liver; F, esophagus; G, stomach; H, colon; I, pancreas.

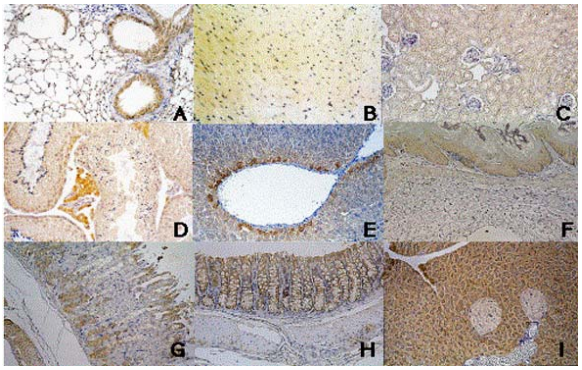

Figure 2. Localization of Aldh2 in mice. The expression of Aldh2 was analyzed as described in Figure 1.

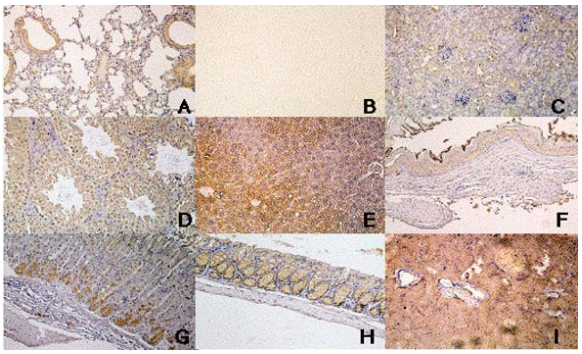

Figure 3. Localization of Cyp1a1 in mice. The expression of Cyp1a1 was analyzed as described in Figure 1.

visualized by ECL (Amersham). The experiments were repeated at least three times.

4. RESULTS

The representative images of immunohistochemical staining of Aldehyde dehydrogenase 1 (Aldh1), Aldh2, Cyp1a1, Cyp2e1 and Cyp4b1 were shown from Figure 1 to Figure 5. The alphabets from A to I indicated organs: A, lung; B, heart; C, kidney; D, testis; E, liver; F, esophagus; G, stomach; H, colon; I, pancreas. Aldh1 and Cyp4b1 were detected around nuclei (perinuclear space). Stained signals of Aldh2, Cyp1a1 and Cyp2e1 were observed in cytoplasm. Aldh1 was strongly expressed in the lung, testis, liver, esophagus, stomach, colon and pancreas (Figure 1). Aldh2 was also expressed in the organs, but with less extent in the esophagus and stomach (Figure 2). Cyp1a1 was strongly expressed in the liver and stomach (Figure 3). Cyp2e1 was strongly expressed in the lung, liver, stomach and colon (Figure 4) and Cyp4b1 was in the lung, testis, liver, esophagus, stomach and pancreas (Figure 5). The results of immunohistochemistry from five mice were summarized with regard to staining pattern and cellular distribution in each organ as shown in Table 3 and 4. In the heart, Cyp1a1 was not detected in this study although it was previously detected by immunohistochemistry (16), Western blot (17), and RT-PCR (18) as seen in Table 5.

Compared with anti-human ALDH2, anti-mouse Aldh2 produced the immunohistochemical images that have staining patterns and intensity very similar to those seen in Figure 2. For instance, anti-mouse Aldh2 produced the immunohistochemical image of the mouse liver (Figure 6B) indistinguishable from that obtained by anti-human ALDH2 (Figure 6A). In the pancreas, however, strongly stained signals by anti-mouse Aldh2 were observed in Langerhans islet (Figure 6D) where no signals were detected by anti-human ALDH2 (Figure 6C), while acinus cells were similarly stained by both antibodies. Because murine Aldh2 is 96% identical to the human enzyme, this observation could be caused by a technical problem that we could not address. The results of comparison between anti-mouse and anti-human ALDH2 were summarized in Table 3.

To examine the effects of Aldh2-disruption on the expression of other enzymes, the expression of the same series of enzymes were investigated using Aldh2 KO mice. As expected, Aldh2 was not detected in all organs of Aldh2 KO mice although only representative data of liver and kidney were shown (Figure 7B and D). The expression patterns of Aldh1, Cyp1a1, Cyp2e1 and Cyp4b1 in 9 organs of Aldh2 KO mice were indistinguishable with those of wild mice (figure not shown). Upon Western blot analysis of subcellular fractions from livers of the wild type and Aldh2 KO mice (Figure 8), the expression level of Cyp2e1 was increased in Aldh2 KO mice although the levels of Aldh1, Cyp1a1 and Cyp4b1 were similar between wild type and Aldh2 KO mice. Although the expression level of Cyp2e1 varied among individual animals of both wild type and Aldh2 KO mice, the increased expression of Cyp2e1 was clearly observed in 7 of 10 livers from Aldh2 KO mice.

The expression of ALDH and CYP in human organs has been reported in many previous studies as seen in Table 6. However, the expression of ALDH1 and ALDH2 has not been reported in the esophagus although the expression of a series of enzymes involved in alcohol metabolism is important to understand the association with esophageal cancer. Therefore, we investigated the expression of the enzymes in the human liver and

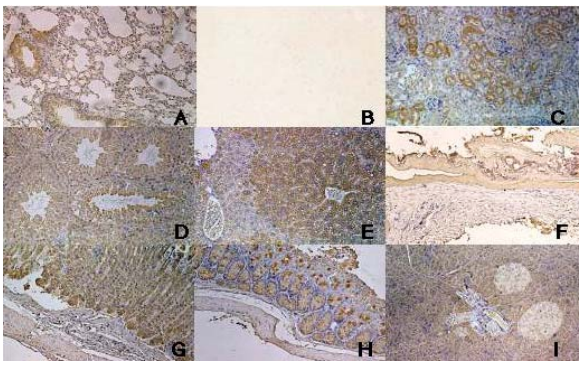

Figure 5. Localization of Cyp4b1 in mice. The expression of Cyp4b1 was analyzed as described in Figure 1.

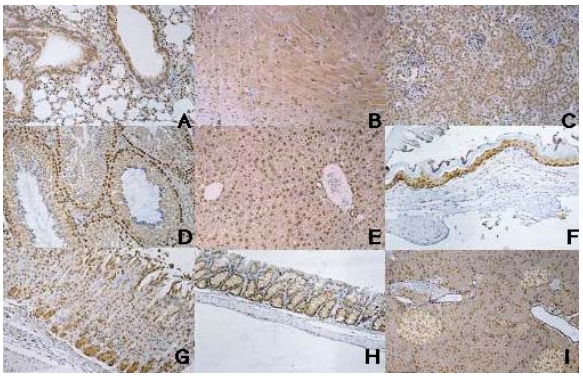

Figure 6. Comparison of anti-human ALDH2 and anti-mouse Aldh2 for immuno-detection of Aldh2 in liver and pancreas from mice. Comparison of anti-human ALDH2 (A and C) and anti-mouse Aldh2 (B and D) was performed by immunochemical staining in liver (A and B) and pancreas (C and D) of mice.

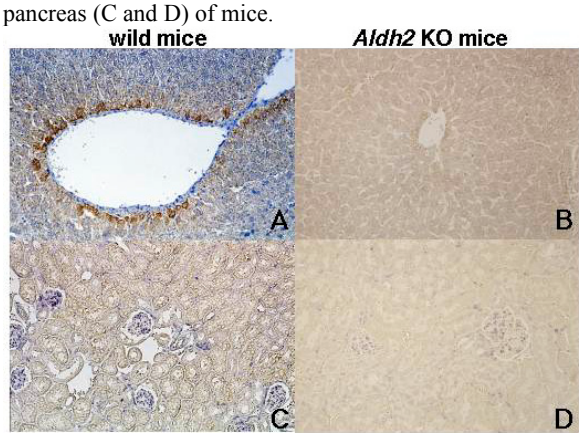

Figure 7. Aldh 2 staining in wild mice and Aldh2 KO mice. Aldh 2 was expressed in liver (A) and kidney (C) of wild type mice, but not detected in liver (B) and kidney (D) of Aldh2 KO mice. Immuno-detection was carried out using anti-mouse Aldh2 antibodies.

esophagus. As shown in Figure 9, ALDH1, ALDH2 and CYP1A1 were detected in the human liver although CYP2E1 was rarely detected. In the human esophagus (Figure 10), ALDH1 and CYP1A1 were detected although CYP2E1 was not detectable. Intriguingly, ALDH2 expression levels largely varied among the human esophageal specimens. One of five specimens from patients with esophageal cancer was judged as negative staining (-), three of five as double positive (++) and one of five as triple positive (+++) in squamous epithelium. The results of Figure 9 and 10 were summarized in Table 7.

5. DISCUSSION

In this study, the expression of Aldh1, Aldh2, Cyp1a1, Cyp2e1 and Cyp4b1 has been systematically investigated in nine organs of wild type and Aldh2 KO mice. All of Aldh and Cyp enzymes were detected in multiple organs, in particular, abundantly expressed in liver and gastrointestinal tract. Aldh1 was ubiquitously found in mice organs although 4 members in Aldh1 subfamily were not distinguished, while the expression of Aldh2 was very low abundant or negative in the heart and kidney. Among Cyp enzymes, Cyp4b1 was detected in all organs examined in this study while Cyp1a1 and Cyp2e1 were not detected in the heart (Table 3 and 4).

As summarized in Table 5, the expression of Cyp1a1 and Cyp2e1 in murine organs has been well investigated by other investigators. However, only the expression of Cyp1a1 has been previously reported in the esophagus (19). In this study, we demonstrated that in the esophagus Aldh1 and Cyp4bl are abundant, the Aldh2 expression is positive, and the Cyp1a1 and Cyp2e1 expression levels are negative-positive. In particular, the first-pass metabolism of ethanol in gastrointestinal tract is of great interest because of the association with oral (10) and esophageal cancers (9). Although the expression of Aldh2 and Cyp2e1 in esophageal epithelium was demonstrated in this study, the effects of chronic alcohol exposure on the expression of Aldh2 and Cyp2e1 remain to be investigated. ADH4 having high K_m and k_{cat} values for ethanol is abundant in the esophagus of mice (20), rats (21) and humans (22). In humans and mice, therefore, ethanol seems to be similarly metabolized to acetaldehyde and acetate, suggesting that the Aldh2-null mouse may be a useful animal model for alcohol-related diseases of esophagus.

Although CYP1A1 and CYP2E1 were previously detected in a variety of organs including esophagus (Table 6) and also in cancer tissues (23), we demonstrated that both ALDH1 and ALDH2 are also expressed in esophageal epithelium in humans. Inactive form of ALDH2 produced by ALDH2*2 allele is a strong risk factor for esophageal (24, 25), oropharyngeal and laryngeal cancers (26-28) in Japan. The local metabolism of ethanol in the esophagus, particularly the accumulation of acetaldehyde caused by lowered or inactivated acetaldehyde dehydrogenase of ALDH1*1/ALDH2*2 or ALDH2*2/ALDH2*2 genotype, may be directly associated with esophageal cancer. In addition to the ALDH2 polymorphism, the inter-individual variation of ALDH2 expression in the esophagus observed in this study (Figure 10) may be also important for consideration of the occurrence of esophageal cancer. Since ALDH2*2 allele is not known to decrease the expression level of the enzyme, this inter-individual variation may be dependent on transcriptional regulation

Table 2. Antibodies and conditions for ALDH and CYP immunohistochemistry

Antigen	Source of primary antibody	Primary antibody	Dilution (folds)
ALDH1	Dr. Weiner [1]	Rabbit anti-human	200
ALDH2	Dr. Weiner [1]	Rabbit anti-human	200
CYP1A1	Commercial antibody [2]	Rabbit anti-human	200
CYP2E1	Commercial antibody [2]	Mouse anti-human	100

[1]: Supplied by Dr. Weiner, University Of Purdue (29), [2] Daiichi Pure Chemicals Co., Ltd.

Table 3. Distribution of Aldh1 and Aldh2 expression in wild mice by immunohistochemistry

Organ	Aldh1 (Rabbit anti-human)	Aldh2 (Rabbit anti-human)	Aldh2 (Rabbit anti-mouse)
Lung	++ Clara cell, II type (Alveolar epithelial cell)	++ Clara cell, II type (Alveolar epithelial cell)	+ Clara cell, II type (Alveolar epithelial cell)
Heart	+ Myocardium	± Myocardium	± Myocardium
Kidney	+ Urinary tubule	± Urinary tubule	± Urinary tubule
Testis	++ Spermatogonium	++ Leydig cell	+ Leydig cell
Liver	++ Liver cell	++ Liver cell (Around central vein)	++ Liver cell (Around central vein)
Esophagus	++ Squamous epithelium, Lamina propria mucosa	+ Squamous epithelium	+ Squamous epithelium
Stomach	++ Fundic gland (Epithelial cell)	+ Glandular neck and base	+ Glandular neck and base
Colon	++ Mucosal layer	++ Mucosal layer	+ Mucosal layer
Pancreas	++ Acinus, Langerhans islet	++ Acinus	+ Langerhans islet
Staining pattern	Perinuclear space	Cytoplasm	Cytoplasm

Negative Positive (±); < 5% Staining, Positive (+); 5% - 10% Staining, Double Positive (++); 10% - 50% Staining

Table 4. Distribution of Cyp1a1, Cyp2e1 and Cyp4b1 expression in wild mice by immunohistochemistry

Organ	Cyp 1a1 (Goat anti-rat)	Cyp 2e1 (Goat anti-rat)	Cyp 4b1 (Rabbit anti-mouse)
Lung	± Bronchial epithelial cell	++ Bronchial epithelial cell, II type (Alveolar epithelial cell)	++ Clara cell, II type (Alveolar epithelial cell)
Heart	-	-	+
Kidney	± Urinary tubule	+ Urinary tubule	+ Urinary tubule
Testis	± Germinal epithelium	+ Spermatozoa	++ Spermatogonium
Liver	++ Around central vein	++ Around central vein	++ Liver cell
Esophagus	± Squamous epithelium	± Squamous epithelium	++ Squamous epithelium
Stomach	++ Glandular base	++ Glandular base	++ Glandular neck, Glandular base
Colon	± Mucosal layer	++ Goblet cell	+ Mucosal layer
Pancreas	± Acinus, Langerhans islet	± Acinus	++ Acinus, Langerhans islet
Staining pattern	Cytoplasm	Cytoplasm	Perinuclear space

Negative-Positive (±); < 5% Staining, Positive (+); 5% - 10% Staining, Double Positive (++); 10% - 50% Staining

Table 5. Aldh and Cyp expressions of mice organs in previous studies

Organ	Aldh1	Aldh2	Cyp1a1	Cyp2e1	Cyp4b1
Lung	IHC (30, 31), mRNA (30, 32)		IHC (19, 33), WB (34-37), mRNA (18, 19, 38, 39)	IHC (40, 41), WB (34, 35, 41), mRNA (41, 42)	mRNA (43)
Heart	mRNA (32)		IHC (16), WB (17), mRNA (18)		
Kidney	mRNA (32)		IHC (19), WB (36, 37), mRNA (18, 19, 39)	IHC (44, 45), WB (44, 46, 47), mRNA (47, 48)	
Testis	mRNA (32)		IHC (19), mRNA (19)	IHC (49), WB (49)	IHC (50), WB (51), mRNA (50)
Liver	mRNA (32)		IHC (19),WB (36, 37), mRNA (18, 19, 38, 39)	IHC (45, 49), WB (46, 49, 52) ,mRNA (42, 52)	mRNA (43)
Esophagus			IHC (19),mRNA (19)		
Stomach	WB (53)	WB (53)	IHC (19),mRNA (19)		
Colon			IHC (19),mRNA (19)	IHC (54),mRNA (54)	
Pancreas			IHC (55)	IHC (55)	

WB: detected by Western blotting analysis, IHC: detected by immunohistochemical staining, mRNA: detected mRNA level, using by reverse-transcriptional (RT)-polymerase chain reaction (PCR), Reference numbers are shown in parenthesis

associated with foods including alcohol or unknown polymorphisms in the transcriptional regulatory region. The expression of CYP2E1 that converts ethanol to acetaldehyde is induced by alcohol while ALDH2 converting acetaldehyde to acetate is decreased by chronic alcohol intake, which may contribute the local accumulation of acetaldehyde in various tissues. Intriguingly, the expression of Cyp2e1 in the liver of Aldh2-null mice was increased compared with that of the wild type mice (Figure 8), suggesting that a

Table 6. ALDH and CYP expression of human organs in previous studies

Organ	ALDH1	ALDH2	CYP1A1	CYP2E1	CYP4B1
Lung	mRNA (56)	mRNA (56)	IHC (57, 58), WB (59, 60), mRNA (59, 60)	IHC (58, 61), mRNA (58, 61, 62)	IHC (61), mRNA (61, 63)
Heart	mRNA (56)	mRNA (56)			
Kidney	WB (64), mRNA (56)	WB (64), mRNA (56)	WB (65), mRNA (66)	WB (65, 67), mRNA (62)	
Testis					
Liver	WB (64) mRNA (56, 68)	WB (64) mRNA (56)	WB (65), mRNA (69, 70)	IHC (71), WB (65, 72), mRNA (70, 73)	
Esophagus			WB (74), IHC (75), mRNA (74, 76)	WB (74), mRNA (74, 77)	mRNA (74)
Stomach			IHC (78), mRNA (76)	IHC (78)	
Colon			WB (65), IHC (79), mRNA (76, 80)	WB (65)	
Pancreas	mRNA (56)	mRNA (56)	IHC (55, 81)	IHC (55, 81)	
Others	Breast-IHC (82)		Brain-IHC, mRNA (83)	Brain-IHC, mRNA (83)	Breast-mRNA (84)

Reference numbers are shown in parenthesis

Table 7. Distributions of ALDH1, ALDH2, CYP1A1 and CYP2E1 expressions in human liver and esophagus by immunohistochemistry

Organ	ALDH1	ALDH2	CYP1A1	CYP2E1
Liver	+++	+++	+++	±
	Liver cell	Around central vein	Liver cell	Liver cell
Esophagus	++	from – to +++	+++	-
	Squamous epithelium Lamina propria mucosa	Squamous epithelium	Squamous epithelium	
Staining pattern	Perinuclear space	Cytoplasm	Cytoplasm	-

negative (0); < no staining, negative-positive (±); < 5% staining, positive (+); 5% - 10% staining, double positive (++); 10% - 50% staining, triple positive (+++); > 50% staining

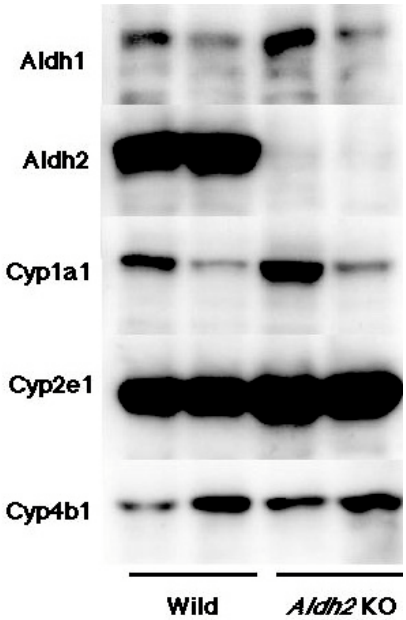

Figure 8. Comparison of Aldh and Cyp in the liver of wild type and Aldh2 KO mice. Proteins (20 μg) of cytosolic (for Aldh1), mitochondria (for Aldh2), and microsomes (for Cyp1a1, Cyp2e1,and Cyp4b1) were displayed on a SDS-polyacrylamide (10%) gel, transferred on to a PVDF membrane, and visualized by ECL kit using the antibodies described in "Materials and Methods".

metabolite(s) produced by Aldh2 might down-regulate the expression of Cyp2e1 gene. If so and also true in human organs, decreased ALDH2 activity might increase the expression of CYP2E1, resulting in the enhancement of local production and accumulation of acetaldehyde. Further studies will be needed to address the relationship among the variation of ALDH2 expression level, ALDH2*2 allele, and esophageal cancer.

6. CONCLUSION

Organ-distribution of Aldh and Cyp enzymes in the mouse has been poorly investigated by previous studies. In the results of this systematical study, we found that: 1) Aldh1 and Aldh2 are expressed in many organs, especially in liver and gastrointestinal tract. 2) Aldh2 and Cyp2e1 are expressed in mice esophageal epithelium. 3) The anti-human ALDH2 cross-reacts with the murine Aldh2 in nine organs indistinguishably with anti-mouse Aldh2, except in Langerhans islet. 4) In Aldh2 KO mice, the expression level of Cyp2e1 was enhanced in the liver. 5) ALDH2 is expressed in human esophageal epithelium with a large variation in the expression level among individuals.

7. ACKNOWLEDGEMENTS

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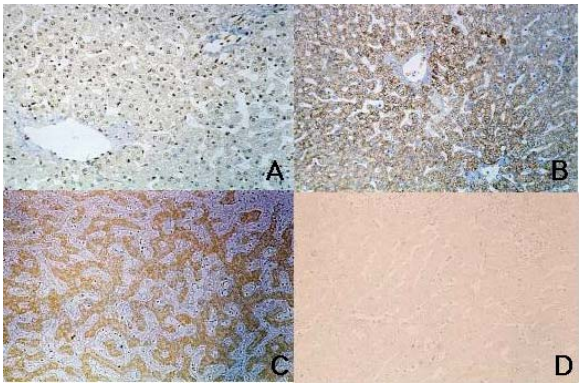

Figure 9. Immunohistochemical detection of ALDH1, ALDH2, CYP1A1 and CYP2E1 in human liver. ALDH1 (A), ALDH2 (B) and CYP1A1 (C) were strongly stained in human liver. CYP2E1 (D) was diagnosed as negative-positive (±).

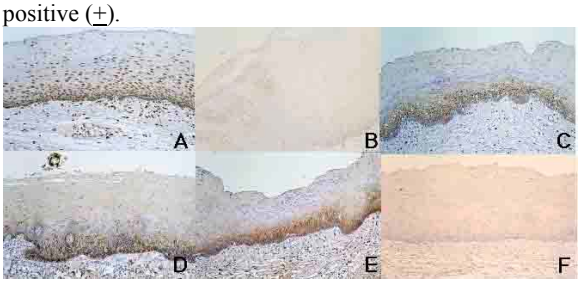

Figure 10. Immunohistochemical detection of ALDH1, ALDH2, CYP1A1 and CYP2E1 in human esophagus. ALDH1 (A) and CYP1A1 (E) were strongly stained in human esophagus. The ALDH2 expression levels of human esophagus varied among individuals (from B to D) and CYP2E1 (F) was not detected.

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Abbreviations: ALDH, aldehyde dehydrogenase; CYP, cytochrome P450; ADH, alcohol dehydrogenase

Key Words: ALDH2, CYP2E1, Esophageal Cancer, Acetaldehyde Dehydrogenase, Alcohol metabolism

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