Gene 437 (2009) 39–44

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Structure and promoter characterization of aldo–keto reductase family 1 B10 gene

Ziwen Liu [a,b,1], Linlin Zhong [a,c,1], Paulette A. Krishack [a], Sarah Robbins [a], Julia X. Cao [a], Yupei Zhao [b], Stephen Chung [d], Deliang Cao [a,*]

[a] Department of Medical Microbiology, Immunology, and Cell Biology, SimmonsCooper Cancer Institute, Southern Illinois University School of Medicine. 913 N. Rutledge Street, Springfield, IL 62702, USA
[b] Department of Surgery, Peking Union Medical College Hospital, Beijing, 100730, PR China
[c] Department of Neurobiology and Department of Anatomy, China Medical University, Shenyang, 110001, PR China
[d] Department of Physiology, the University of Hong Kong, Hong Kong, PR China

ARTICLE INFO

Article history:
Received 14 October 2008
Received in revised form 28 January 2009
Accepted 9 February 2009
Available online 21 February 2009

Received by T. Sekiya

Keywords:
AKR1B10
Aldose reductase-like-1
ARL-1
Promoter
Gene structure

ABSTRACT

Aldo–keto reductase family 1 member B10 (*AKR1B10*) is overexpressed in human hepatocellular carcinoma, lung squamous carcinoma, and lung adenocarcinoma in smokers. Our recent studies have showed that *AKR1B10* plays a critical role in the growth and proliferation of cancer cells by detoxifying reactive carbonyls and regulating fatty acid biosynthesis. However, little is known about the regulatory mechanisms of *AKR1B10* expression. In this study, we determined the structure of *AKR1B10* gene and characterized its promoter. The results demonstrated that *AKR1B10* consists of 10 exons and 9 introns, stretching approximately 13.8 kb. A 5′-RACE study determined the transcriptional start site of *AKR1B10* at 320 bp upstream of the ATG translational start codon. A TATA-like (TAATAA) and a CAAT box are present from −145 to −140 bp and −193 to −190 bp upstream of the transcriptional start site, respectively. Motif analysis recognized multiple putative oncogenic and tumor suppressor protein binding sites in the *AKR1B10* promoter, including c-Ets-1, C/EBP, AP-1, and p53, but osmolytic response elements were not found. A −4091 bp of the 5′-flanking fragment of the *AKR1B10* gene was capable of driving GFP and luciferase reporter gene expression in HepG2 cells derived from human hepatocellular carcinoma; progressive 5′-deletions revealed that a −255 bp fragment possesses full promoter activity.

1. Introduction

Aldo–keto reductase family 1 member B10 (AKR1B10), also known as aldose reductase-like-1 (ARL-1), is a novel protein identified from human hepatocellular carcinoma (HCC) (Cao et al., 1998; Hyndman and Flynn, 1998). This protein belongs to the aldo–keto reductase superfamily, a protein cluster implicated in osmolytic regulation, carbonyl detoxification, cellular carcinogenesis, and cancer therapeutics (Ko et al., 1997; Lee et al., 2001; Crosas et al., 2003; Hyndman et al., 2003; Jin et al., 2006). *AKR1B10* is primarily expressed in the adrenal gland, colon and small intestine with low levels in the liver, thymus, prostate and testis (Cao et al., 1998; Hyndman and Flynn, 1998), but overexpressed in 54% of HCC, 84.4% of lung squamous cell carcinoma, and 29.2% of lung adenocarcinoma in smokers, potentially serving as a diagnostic and/or prognostic marker (Cao et al., 1998; Fukumoto et al., 2005; Penning, 2005).

AKR1B10 is a monomeric enzyme that efficiently catalyzes the reduction of carbonyls with NADPH as a co-enzyme (Cao et al., 1998; Gallego et al., 2007). This reaction converts highly reactive aldehydic and ketonic groups into hydroxy groups, protecting cells against carbonyl toxicity. Our recent studies have demonstrated that targeting expression of *AKR1B10* gene in colorectal cancer cells (HCT-8) and transformed human embryonic kidney cells (293T) significantly affected the cell proliferation, clonogenic growth, and susceptibility to reactive carbonyls, such as acrolein and crotonaldehyde (Yan et al., 2007; Zu et al., 2007). AKR1B10 also shows strong enzymatic activity toward all-*trans*-retinal, 9-*cis*-retinal, and 13-*cis*-retinal, converting them to retinols (Crosas et al., 2003). This reaction may diminish cellular retinoic acid, a signaling molecule regulating cell proliferation and differentiation (Dragnev et al., 2000; Penning, 2005).

In H-*ras* transformed human mammary epithelial cells and colorectal cancer cells (HCT-8), AKR1B10 affects *de novo* synthesis of fatty acids by mediating acetyl-CoA carboxylase-α (ACCA) degradation through the ubiquitination–proteasome pathway (Ma et al., 2008). ACCA is a rate-limiting enzyme in long chain fatty acid synthesis, catalyzing malonyl-CoA formation by ATP-dependent carboxylation of acetyl-CoA (Witters et al., 1994; Zang et al., 2005). Long chain fatty acids are the building blocks of biomembranes and the precursors of lipid second messengers, thus being critical to cell proliferation, migration, signal transduction, and intracellular trafficking (Manes et al., 1999; Simons and Toomre, 2000; Rouquette-Jazdanian et al., 2002; Swinnen

Abbreviations: ACCA, acetyl-CoA carboxylase-α; AKR1B1, aldo-keto reductase family 1 B1; AKR1B10, aldo-keto reductase family 1 B10; ARL-1, aldose reductase-like-1; HCC, hepatocellular carcinoma; GFP, green flourescent protein; RACE, rapid amplification of cDNA ends; TdT, dNTP transferase.

* Corresponding author. Tel.: +1 217 545 9703; fax: +1 217 545 9718.
E-mail address: dcao@siumed.edu (D. Cao).

0378-1119/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2009.02.007

Table 1
Primer sequences used for promoter subcloning.

Primer	Sequences	Location
P1	5′- CTAATCTGTCACCTTGGAGG - 3′	−4091 bp
P2	5′- CTAAAAAAGATATCCCTTCTCACTGATTC - 3′	−2067 bp
P2341	5′- AGGACCTCTG AACAACTGCG TG - 3′	−1776 bp
P3056	5′- GAAGTATAAGATTTTTCACTCATAG - 3′	−1061 bp
P3862	5′- CCCTACCTTCCAACTTTTGGCTG - 3′	−255 bp
P320a (R)[a]	5′- GAAT CATTTCTGCA CCAACC - 3′	+320 bp

[a] Reverse primer, used to pair with all forward primers for amplification.

et al., 2004; Swinnen et al., 2006). In breast cancer cells, *ACCA* knockdown induced by small interfering RNA resulted in cell cycle arrest and apoptosis (Chajes et al., 2006). Therefore, AKR1B10 may play a critical role in the development and progression of cancer through detoxifying intracellular cytotoxic carbonyls, mediating retinal metabolism, and regulating fatty acid biosynthesis. However, the regulatory mechanisms of *AKR1B10* expression remain unclear. This study determined *AKR1B10* gene structure and characterized its promoter, aiding in the understanding of its expression regulation.

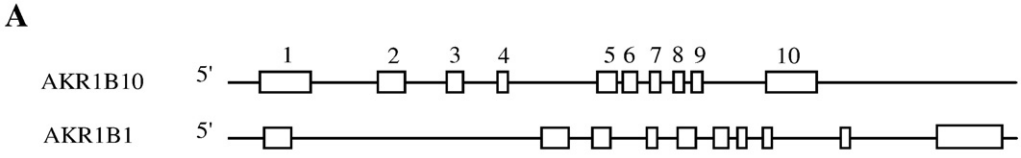
2. Materials and methods

2.1. Cell culture

HepG2 (human hepatocellular carcinoma) cells were purchased from American Type Culture Collection (Manassas, VA) and maintained in RMPI 1640 medium (Hyclone, UT) containing 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C, 5% CO_2.

2.2. 5′-RACE (rapid amplification of cDNA ends)

Total RNA was extracted using Trizol® reagent (Invitrogen, CA) from HepG2 cells and hepatocellular carcinoma tissues (collected via Co-operative Human Tissue Network sponsored by National Cancer Institute) as previously described (Cao et al., 2005). Contaminated genomic DNA was removed by DNase I at 37 °C for 30 min in 200 μl mixture containing 20 μg total RNA, 20 U RNase-free DNase I, and 50 U RNase inhibitor (Invitrogen, CA), followed by incubation with proteinase K (2.0 mg/ml in 0.5% SDS) and purification with phenol/chloroform extraction. The first strand cDNA was synthesized at 42 °C for 1 h in 40 μl mixture consisting of 10 pmol gene specific primer (refer to Fig. 2), 200 μM dNTP, 10 μg total RNA, 8 μl 5× reaction buffer, 4 μl 0.1 M DTT, and 400 U Superscript II® retrotranscriptase (RTase). The RNA strand was removed by RNase H (0.5 U/μl, final) at 55 °C for 10 min, and cDNA was purified with GLASSMAX DNA isolation spin cartridge. Poly (dC) tail was added by terminal dNTP transferase (TdT, Invitrogen, CA) at 37 °C for 10 min in reaction mixture composed of 10 U TdT and 200 μM dCTP, followed by heat inactivation at 65 °C for 15 min. The dC-tailed cDNA was amplified in 50 μl PCR mix containing 5 μl dC-tailed cDNA, 10 pmol gene specific primer, 10 pmol anchor primer (Invitrogen,



B

Gene	3' end exon	5' Splice donor	Intron (size)	3' Splice acceptor	5' end exon	Exon (size)
AKR1B10					acagt	..Ex 1 (385 bp)...
AKR1B1					agcgg	..Ex 1 (163 bp)...
AKR1B10	ggaag	**GT**AAA	..Int 1 (2665 bp)...	TTC**AG**	tctcc	..Ex 2 (168 bp)...
AKR1B1	ggaag	**GT**AGG	..Int 1 (7243 bp)...	TTC**AG**	tcccc	..Ex 2 (168 bp)...
AKR1B10	gcaag	**GT**GCA	..Int 2 (1297 bp)...	TGC**AG**	ttgtg	..Ex 3 (117 bp)...
AKR1B1	gcaag	**GT**ATC	..Int 2 (683 bp)...	CTT**AG**	ctgtg	..Ex 3 (117 bp)...
AKR1B10	tcaag	**GT**TTA	..Int 3 (979 bp)....	CAC**AG**	tctgg	..Ex 4 (78 bp)....
AKR1B1	ttaag	**GT**ATG	..Int 3 (988 bp)....	GAC**AG**	cctgg	..Ex 4 (78 bp)....
AKR1B10	gggag	**GT**AGG	..Int 4 (3568 bp)...	GAT**AG**	gccat	..Ex 5 (123 bp)...
AKR1B1	gggcg	**GT**AAG	..Int 4 (678 bp)...	TGT**AG**	gccat	..Ex 5 (123 bp)...
AKR1B10	accag	**GT**AAA	..Int 5 (278 bp)....	TGC**AG**	gttga	..Ex 6 (107 bp)...
AKR1B1	accag	**GT**AAA	..Int 5 (503 bp)....	TGC**AG**	attga	..Ex 6 (107 bp)...
AKR1B10	ccttg	**GT**GAG	..Int 6 (422 bp)....	CCT**AG**	ggcca	..Ex 7 (82 bp)....
AKR1B1	ccctg	**GT**GAG	..Int 6 (325 bp)....	CTC**AG**	ggcca	..Ex 7 (82 bp)....
AKR1B10	cccag	**GT**GCC	..Int 7 (532 bp)....	CAT**AG**	gttct	..Ex 8 (84 bp)....
AKR1B1	cccag	**GT**ACA	..Int 7 (598 bp)....	CAC**AG**	gtcct	..Ex 8 (84 bp)....
AKR1B10	ttcag	**GT**AAG	..Int 8 (657 bp)...	TCC**AG**	gtctt	..Ex 9 (83 bp)....
AKR1B1	ttaag	**GT**AAG	..Int 8 (1977 bp)...	TTT**AG**	gtctt	..Ex 9 (83 bp)....
AKR1B10	ttgca	**GT**AAG	..Int 9 (2029 bp)...	TGC**AG**	atcct	..Ex 10 (360 bp)..
AKR1B1	ttgag	**GT**GAG	..Int 9 (2467 bp)...	GAC**AG**	ctgta	..Ex 10 (409 bp)..
AKR1B10	atcaataaaaaaaataataa (Poly A signal)					
AKR1B1	ctcaaataaaaactgctttt (Poly A signal)					

Fig. 1. Gene structures of *AKR1B10* and *AKR1B1*. (A) Scaled representation of *AKR1B10* and *AKR1B1* genes. Open boxes indicate the exons numbered on the top. (B) Nucleotide sequences in intron–exon borders. *AKR1B10* and *AKR1B1* genes both contain ten exons and nine introns following a typical GT/AG (bolded) rule at donor and acceptor sites.