The International Journal of Biochemistry & Cell Biology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

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

A natural antisense transcript regulates *acetylcholinesterase* gene expression via epigenetic modification in Hepatocellular Carcinoma

Q1 Qiliang Xi[a], Ning Gao[b], Xuejin Zhang[a], Bo Zhang[a], Weiyuan Ye[a], Jun Wu[a], Xuejun Zhang[a,*]

[a] *State Key Laboratory of Cell Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Graduate School of the Chinese Academy of Sciences, 320 YueYang Road, Shanghai 200031, PR China*

[b] *State Key Laboratory of Molecular Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Graduate School of the Chinese Academy of Sciences, 320 YueYang Road, Shanghai 200031, PR China*

ARTICLE INFO

Article history:
Received 26 March 2014
Received in revised form 3 September 2014
Accepted 9 September 2014
Available online xxx

Keywords:
Acetylcholinesterase
AChE antisense RNA
Epigenetic modulation
Apoptosis
Cis-platin
Mitomycin C

ABSTRACT

In recent years, widespread antisense transcripts have been identified systematically in mammalian cells and are known to regulate gene expression, although their functional significance remains largely unknown. Previous work has identified that *acetylcholinesterase* (AChE) is expressed aberrantly in various malignant tumors and function as a tumor growth suppressor. However, the mechanism of *AChE* gene regulation in tumors remains unclear. In this study, we show that the *AChE* antisense RNA (*AChE-AS*) play an important role in AChE expression regulation. An inverse relationship was identified between *AChE-AS* and AChE expression in hepatocellular carcinoma and hepatoma cells. The silenced *AChE-AS* corresponds to elevated expression of AChE. Furthermore, we demonstrated that reduced *AChE-AS* increased H3K4 methylation and decreased H3K9 methylation in the *AChE* promoter region. As expected, elevated AChE levels induced by inhibition of *AChE-AS* enhanced anticarcinogen-induced apoptosis. These observations demonstrated that *AChE-AS* modulates AChE expression and exerts an anti-apoptotic effect through direct repression of AChE expression in HCC cells. Thus, natural antisense RNA may play an important role in AChE regulation via affecting the epigenetic modification in the *AChE* promoter region.

© 2014 Published by Elsevier Ltd.

1. Introduction

The classical function of *acetylcholinesterase* (AChE, EC3.1.1.7) is the termination of transmission at cholinergic synapses and neuromuscular junctions by hydrolyzing the neurotransmitter acetylcholine (ACh) (Taylor and Radic, 1994; Soreq and Seidman, 2001; Silman l, 2005). There are three different alternatively spliced forms of *AChE* in humans: *S-AChE* (synaptic *acetylcholinesterase*), *R-AChE* (read-through *acetylcholinesterase*) and *E-AChE* (erythrocytic *acetylcholinesterase*) (Grisaru et al., 1999). AChE has several non-canonical functions in addition to the classic function of this enzyme. For instance, recent reports reveal that increased expression of AChE induces apoptosis in a number of cell lines and functions as a tumor suppressor gene (Yang et al., 2002; Zhang et al., 2002; Zhao et al., 2011; Lu et al., 2013).

Several reports document modulated expression of AChE in a variety of tumors, such as ovarian, breast, brain, colon, renal and non-small cell lung cancers as well as hepatocellular carcinomas (Ruiz-Espejo et al., 2002; Vidal, 2005; Montenegro et al., 2006; Syed et al., 2008; Munoz-Delgado et al., 2010; Zhao et al., 2011; Lu et al., 2013; Zakut et al., 1990). Recent research in our laboratory demonstrated that microRNA-212 inhibited the expression of AChE and its expression was inversely correlated with AChE in NSCLC cell lines and a subset of tumor samples (Lu et al., 2013). Meanwhile, Sailaja and colleagues demonstrated that the epigenetic state of the AChE affects AChE expression (Sailaja et al., 2012).

In recent years, a growing number of naturally occurring antisense transcripts (NATs) have been documented (*Kiyosawa et al., 2003; Katayama et al., 2005; Ge et al., 2006; He et al., 2008*). Antisense transcripts are from opposite DNA strands either *in cis* at the same genomic locus or *in trans* at separate loci (Lavorgna et al., 2004), and partially complementary to other RNAs (Lavorgna et al., 2004; Zhang et al., 2006). In the human genome, it is

Abbreviations: AChE, *acetylcholinesterase*; *S-AChE*, synaptic *acetylcholinesterase*; *R-AChE*, read-through *acetylcholinesterase*; *E-AChE*, erythrocyte *acetylcholinesterase*; AZA, 5-Aza-2′-deoxycytidine; TSA, trichostatin A; CDDP, cisplatin; MMC, mytomycin C; HCC, hepatocellular carcinoma; ChIP, chromatin immunoprecipitation; RNAi, RNA interference; siRNA, small interfering RNA; siNC, negative siRNA; TUNEL, TdT-mediated dUTP nick-end-labeling; UTR, untranslated region.

* Corresponding author. Tel./fax: +86 21 54921403.
E-mail address: xjzhang@sibcb.ac.cn (X. Zhang).

http://dx.doi.org/10.1016/j.biocel.2014.09.012
1357-2725/© 2014 Published by Elsevier Ltd.

G Model
BC 4435 1–10
ARTICLE IN PRESS

predicted that 22% (5880) of the human transcription clusters form sense-antisense (SA) pairs (Chen et al., 2004) and at least 10–40% of polypeptide-encoding genes are associated with antisense transcription (Shendure and Church, 2002; Yelin et al., 2003; Katayama et al., 2005; He et al., 2008). NATs have been shown to be involved in many biological processes, such as genomic imprinting (Lee et al., 1999), X chromosome inactivation (Wutz, 2007), regulation of gene expression (Yu et al., 2008; Mahmoudi et al., 2009), cancer and other diseases (Korneev and O'Shea, 2005; Yu et al., 2008). NATs can affect gene expression at various levels including transcription, RNA processing and transport, and translation (Beiter et al., 2009; Spigoni et al., 2010).

However, NATs regulate gene expression via distinct mechanisms. First, NAT transcription disturbs the sense transcription by affecting polymerase activity (Crampton et al., 2006). Second, it may affect the splicing, stabilization and half-life of its sense partner by forming complementary double-strand RNA (Hastings et al., 2000; Beltran et al., 2008; Tam et al., 2008). Third, it can modulate the methylation state and influence chromatin remodeling of the chromosome (de Bustros et al., 1988; Sleutels et al., 2002; Okamoto et al., 2004). Finally, its interaction with some proteins can affect the translation of mRNA (Ebralidze et al., 2008).

In this study, we demonstrated the existence of a NAT with the capacity to modulate *AChE* gene expression. Furthermore, we identified an inverse relationship between *AChE* antisense (*AChE-AS*) and *AChE* sense expression in hepatocellular carcinoma and hepatoma cells. These observations indicated that *AChE-AS* participates in the regulation of AChE expression. Furthermore, we demonstrated that the sense/antisense regulation was due to histone modification of chromatin in the *AChE* promoter and was involved in the elevated expression of AChE in cells treated with apoptosis-inducing agents. Thus, the sense-antisense interaction of *AChE* may play an important role in tumorigenesis and development.

2. Materials and methods

2.1. Cell culture and reagents

Cell lines LO2, QSG7701, SMMC7721, Bel7404, Huh7, HepG2, QGY7703 and Hep3B were a generous gift from Prof. Lijian Hui (Shanghai Institute of Biochemistry and Cell Biology, China). Cell lines were cultured in media as recommended by the American Type Culture Collection (ATCC). Cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2. Cisplatin (CDDP), mitomycin C (MMC) and trichostatin A (TSA) (Sigma–Aldrich, St. Louis, MO, USA) were dissolved with dimethyl sulfoxide (DMSO). 5-Aza-2′-deoxycytidine (AZA) (Sigma–Aldrich, St. Louis, MO, USA) was dissolved with phosphate-buffered saline (PBS). G418 (ShineGene Molecular Biotech, Inc.) was dissolved with 10% 1 M HEPES.

2.2. Tissue specimens

Human hepatocellular cancer specimens were collected from patients undergoing surgical resection in the First Affiliated Hospital of Sun Yat-sen University, Second Affiliated Hospital Sun Yat-sen University and Sun Yat-sen University Cancer Center. Fresh hepatocellular carcinomas and paired adjacent normal tissues were immediately snap-frozen in liquid nitrogen and preserved in −80 °C until use and their histological type was further confirmed using standard hematoxylin and eosin staining. Informed consent was obtained from all patients.

2.3. Plasmid construction

The pAChE and pAChE-AS constructs were based on the pIRES2-EGFP vector (Clontech). First, the *CMV* promoter in the pIRES2-EGFP vector was replaced by the *SV40* poly(A) sequence. The DNA fragment of *SV40* poly(A) was obtained by PCR using the forward primer F; 5′-CTAGCTAGCTGATCATAATCAGCCATACCAC-3′, and reverse primer R; 5′-CCGATTAATTAAGATACATTGATGAGTTTGGAC-3′, and was cloned into the NheI/AseI sites. For pAChE, the human *AChE* promoter (Ben Aziz-Aloya et al., 1993) was ligated into the BglII and EcoRI sites of the pIRES2-EGFP vector. The human *AChE* promoter was obtained by PCR using the forward primer F; 5′-CCGAGATCTGTACCGAGCTCTTACGCGTGCG-3′, and reverse primer R; 5′-CCGGAATTCCAACAGTACCGGAATGCCAAGC-3′. For pAChE-AS, the amplified plasmid *CMV* promoter was ligated into the SalI and EcoRI sites of the pAChE construct. Primers for the CMV promoter are the forward primer F; 5′-CCGG-AATTCATAGTAATCAATTACGGGGTCATTAGTTC-3′, and reverse primer R; 5′-ACGCGTCGACGATCTGACGGTTCACTAAACCAGCT-3′. For pEGFP-N1-AChE, the full-length human *AChE* complementary DNA (cDNA) sequence (GenBank accession No. M55040) was subcloned into the BglII and EcoRI sites of the pEGFP-N1 (Clontech, Mountain View, CA, USA) vector. The primers used are the forward primer F; 5′-CCGAGATCTATGAGGCCCCCGCAGTGTCT-3′, and reverse primer R; 5′-CCGGAATTCTCACAGGTCTGAGCAGCGATCC-3′.

2.4. RNA interference

The stealth RNAi oligonucleotides specifically targeting the *AChE-AS* were designed and synthesized by GenePharma (Shanghai). AChE-AS-siRNA1, 5′-GCUACGAGAUCGAGUUCAUTT-3′, AChE-AS-siRNA2, 5′-GUGUCUGCUACCAAUAUGUTT-3′ and AChE-AS-siRNA3, 5′-GUCCUGCAUUACACAGACUTT-3′.

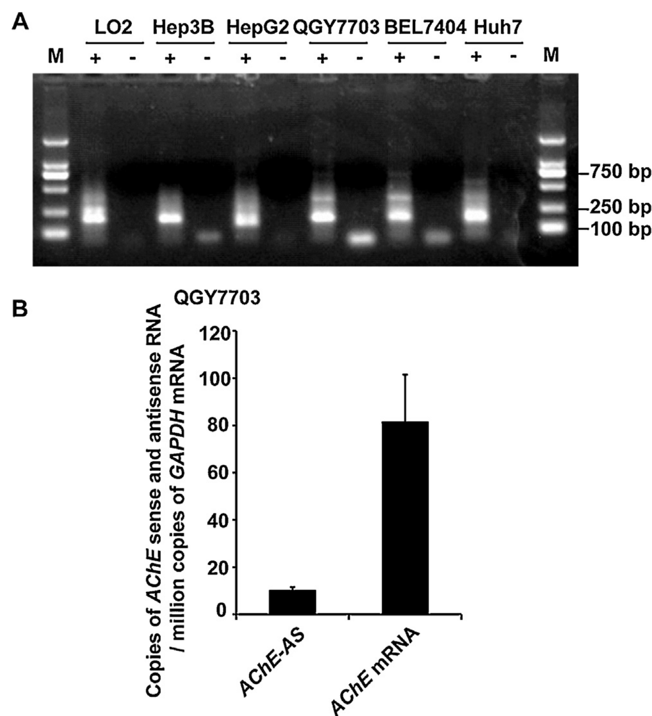

Fig. 1. The expression of *AChE-AS* antisense in liver normal and cancer cell lines. (A), Native *AChE-AS* transcripts were amplified from the cDNA reverse-transcribed from RNA by a strand-specific primer from liver cell lines LO2 and HCC cell lines Hep3B, HepG2, QGY7703, BEL7404 and Huh7. "+", RT-PCR with reverse transcriptase; "−", controls without reverse transcriptase. M is a DL2000 ladder. (B), qRT-PCR analysis showing *AChE* mRNA and *AChE-AS* levels in QGY7703 cells. Each experiment was repeated a minimum of three times.

Please cite this article in press as: Xi Q, et al. A natural antisense transcript regulates *acetylcholinesterase* gene expression via epigenetic modification in Hepatocellular Carcinoma. Int J Biochem Cell Biol (2014), http://dx.doi.org/10.1016/j.biocel.2014.09.012

Download English Version:

https://daneshyari.com/en/article/8323077

Download Persian Version:

https://daneshyari.com/article/8323077

Daneshyari.com