Neuroscience Letters 662 (2018) 91–97

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research article

The correlation between DNA methylation and transcriptional expression of human dopamine transporter in cell lines

Desheng Zhai[a,1], Songji Li[b,1], Gaopan Dong[b], Dushuang Zhou[b], Yuxin Yang[c], Xin Wang[d], Ying Zhao[b,*], Yunlei Yang[b,e,f,*], Zhicheng Lin[d]

[a] School of Public Health, Henan Collaborative Innovation Center of Molecular Diagnosis and Laboratory, Xinxiang Medical University, Xinxiang 453003, China
[b] School of Pharmacy, Xinxiang Medical University, Xinxiang 453003, China
[c] The First Affiliated Hospital, Xinxiang Medical University, Weihui 453100, China
[d] Department of Psychiatry and Department of Neurology, Harvard Medical School, McLean Hospital, Belmont, MA, 02478, USA
[e] Department of Neuroscience & Physiology, State University of New York Upstate Medical University, Syracuse, NY 13210, USA
[f] Disciplinary Group of Psychology and Neuroscience, Xinxiang Medical University, Xinxiang 453003, China

ARTICLE INFO

Keywords:
DNA methylation
Human dopamine transporter
Cell-specific expression
Epigenetics

ABSTRACT

This study aims to investigate the relationship between DNA methylation and expression of human dopamine transporter (*hDAT*). We examined methylation status of *hDAT* in cells with various *hDAT* expression levels, including two dopaminergic neural cell lines (SK-N-AS and SH-SY-5Y) and one non-dopaminergic cell line (HEK293) by bisulfite sequencing PCR(BSP). The effects of DNA methyltransferase inhibitor 5-aza-dC or/and histone deacetylase inhibitor (HDACi, sodium butyrate, NaB) on the DNA methylation status and mRNA expression levels of *hDAT* were examined. The results revealed marked hypomethylation of the two promoter regions (−1214 to −856 bp and −48 to 439 bp, the first base of exon 1 was taken as +1 bp)of *hDAT* in SK-N-AS (4.7% ± 2.0 ^{mC} and 3.5% ± 1.0 ^{mC}, respectively) compared with SH-SY-5Y (88.0% ± 4.4%^{mC} and 81.1% ± 8.8%^{mC}) and HEK293 (90.7% ± 2.4 ^{mC} and 84.4% ± 8.6% ^{mC}) cell lines, indicating a cell-specific methylation regulation of *hDAT*. 5-aza-dC and NaB decreased hypermethylation,while increase *hDAT* expression in SH-SY-5Y cells and recovered *hDAT* mRNA expression in HEK293 cells. DNA methylation enabled the cell-specific differential expression of the *hDAT* gene. *hDAT* silencing was reversed by the introduction of DNA hypomethylation via 5-aza-dC or/and NaB.

1. Introduction

The human dopamine transporter (*hDAT*, gene symbol: *SLC6A3*), an important monoamine transporter, is involved in the pathogenesis of schizophrenia, addiction, and Parkinson's disease [1–3]. The activity and levels of expression of *hDAT* provide vital determinants of dopamine function. DAT acts to terminate dopaminergic neurotransmission by re-uptake of dopamine into presynaptic neurons [4,5]. Knockdown or knockout of the DAT gene leads to DA dyshomeostasis and contributes to several behavioral abnormalities including hyperlocomotion, and cognitive and behavior deficits [6,7]. Thus, appropriate regulation of *hDAT* expression is vital to maintain dopamine homeostasis, and meanwhile disruption of *hDAT* expression and function can lead to neurological and psychiatric disorders [8–11].

Current research focuses on illuminating the mechanisms regulating *hDAT* in the brain and on identification of the genetic determinants that are involved in *hDAT* regulation [4,9,11–14]. It is known that epigenetic modifications play an important role in the regulation of gene expression [15,16]. Epigenetic modifications are defined as mechanisms that regulate gene expression without altering the underlying DNA sequence [16,17]. As such, tissue specific epigenetic regulation determines phenotypic differences between cells [15]. Epigenetic modifications mainly include DNA methylation, histone modifications and non-coding RNA-mediated changes of gene transcripts [16,17]. Bioinformatic analyses revealed that *hDAT* are sensitivity to regulation by epigenetic mechanisms [18]. The core promoter is GC-rich, suggesting *hDAT* may be regulated through DNA methylation [18], (also see Fig. 1A). There are growing evidences from globe DNA methylation microarray profiles that support roles for DNA methylation regulation of *hDAT* in neuropsychiatric disorders [19–21] Several researches have explored the relationship between various neuropsychiatric disorders and DNA methylation [22–33], and give clinical implication of *hDAT*

* Corresponding authors.
E-mail addresses: zhaoyingxxmc@163.com (Y. Zhao), yangyun@upstate.edu (Y. Yang).
[1] Both authors contributed equally to this work.

http://dx.doi.org/10.1016/j.neulet.2017.10.013
Received 7 February 2017; Received in revised form 7 October 2017; Accepted 9 October 2017
Available online 10 October 2017
0304-3940/

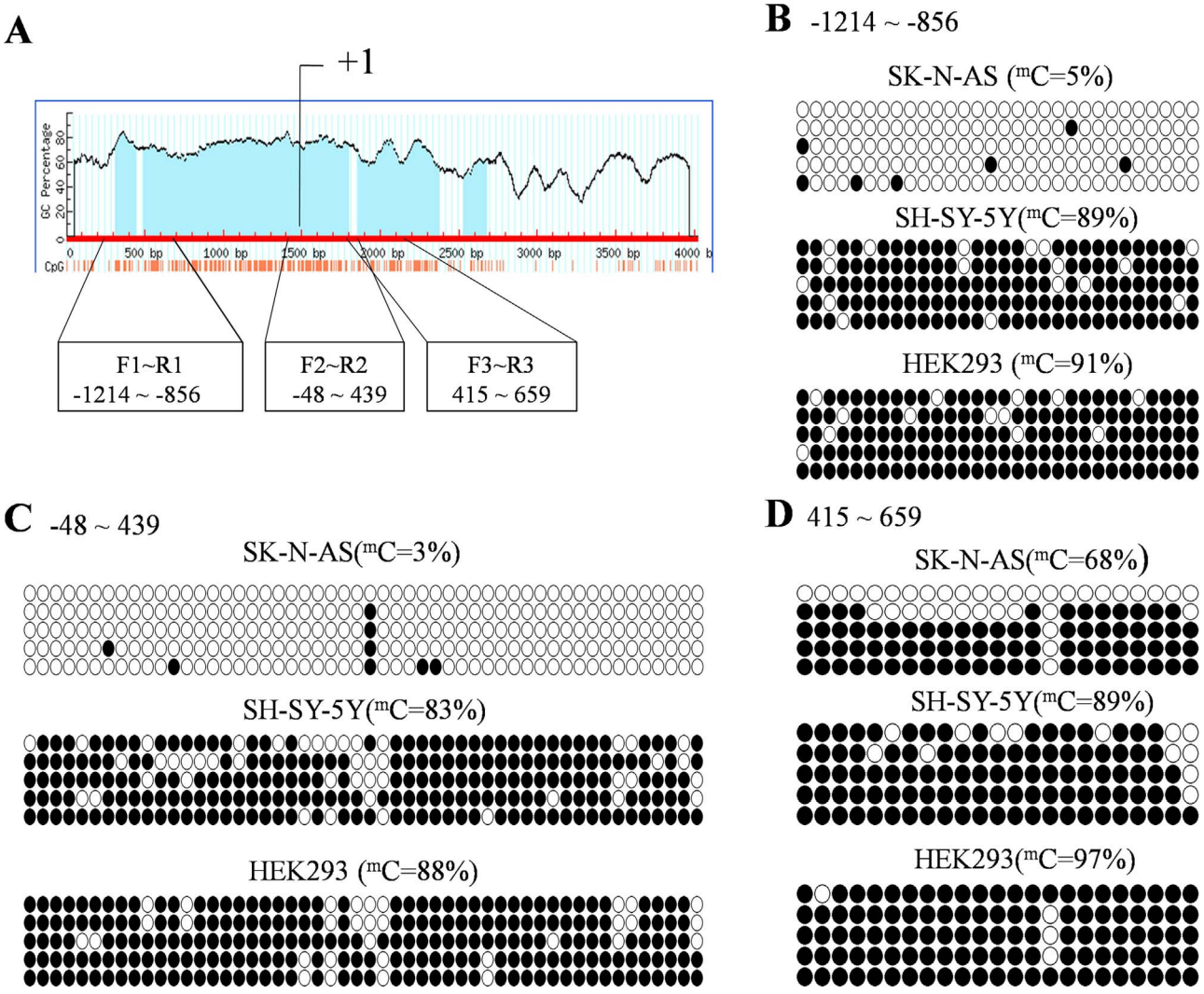

Fig. 1. (A). Genomic CpG island features of the *hDAT* gene locus. The bottom panel shows the locations of three pairs of primers used for bisulfite sequencing of PCR. The first base of exon 1 as +1 bp. (B, C, D). Typical DNA methylation status of the *hDAT* promoter (from −1214 to −856 bp, panel B), (from −48 to 439 bp, panel C) and the *hDAT* coding region (from 415 to 659 bp, panel D) in SK-N-AS, SH-SY-5Y and HEK293 cells. The filled or open blocks represent the methylated or unmethylated CpG sites, respectively. Each line represents one clone.

methylation. Recently, Green and his colleague demonstrated the contribution of DNA methylation in regulating *hDAT* expression in SK-N-AS cell lines, using pharmacologic (inhibition of DNA methyltransferase activity) and genetic approaches (siRNA knockdown of DNMT1) [34]. It is assumed that epigenetic mechanisms, especially DNA methylation is responsible for orchestrating complex transcriptional regulation of *hDAT* expression. Consequentially, evaluation *hDAT* methylation has become a vital means for identifying the mechanism of *hDAT* regulation.

The epigenetic mechanism of regulation of *hDAT* expression, however, has not been evaluated rigorously. As such, *hDAT* is mainly expressed in dopaminergic neurons, other neurons has little or no *hDAT* expression. Therefore, we carried out this work mainly to test the hypothesis that DNA methylation plays an important role in the cell-specific expression of *hDAT*.

2. Materials and methods

2.1. Cell culture and epigenetic drug treatment

Human dopaminergic neuroblastoma SK-N-AS (ATCC-CRL-2137), SH-SY-5Y (ATCC-CRL-2266), and human embryonic kidney cells HEK293 (ATCC-CRL-11268) (American Type Culture Collection, Manassas, VA; USA) were maintained in a Dulbecco's modified Eagle's medium supplemented with heat-inactivated 10% fetal bovine serum and 50 U/ml penicillin, 50 mg/ml streptomycin (Beyotime biotechnology,Shanghai,China) at 37 °C in a humidified 5% CO_2 atmosphere. 5-aza-2′-deoxycytidine (5-aza-dC) and sodium butyrate (NaB) were purchased from Energy Chenical (Shanghai, China). 5-aza-dC solution was made freshly in dimethyl sulfoxide(DMSO) prior to each experiment. Cells were exposed to 5 μmol/L 5-aza-dC for 72 h (h) or/and 5 mmol/L sodium butyrate (NaB) for only 24 h(h). Mock-treated cells were given vehicle only. All experiments were done in duplicate and repeated at least three times.

2.2. RNA preparation, cDNA synthesis, and quantitative real-time PCR

Cells were treated as indicated, total cellular RNA was extracted using RNAiso Plus according to the instructions of the manufacturer (Takara BIO INC. Dalian, China). A reverse transcription reaction was performed using 0.5 μg of total RNA with HiScript 1 st Strand cDNA Synthesis Kit (Vazyme, Biotech, USA) according to the manufacture's instructions. Each Real-time PCR reaction included 10 μl of 2 × SYBR Green PCR Mastermix (Takara Bio Inc. Dalian, China), 0.2 μl of forward primers, 0.2 μl of reverse primers, 2 μl of cDNA solution and 7.6 μl of H_2O, with the following parameters: 95 °C for 30 s, followed by 40