Available online at www.sciencedirect.com
ScienceDirect
J. Genet. Genomics 35 (2008) 153−161
JOURNAL OF GENETICS AND GENOMICS
www.jgenetgenomics.org

The ethanol response gene *Cab45* can modulate the impairment elicited by ethanol and ultraviolet in PC12 cells

Yunfeng Zhu [a, *], Quanli Wang [b], Wangru Xu [a], Sha Li [a]

[a] *Department of Oncology in Beijing 307 Hospital, Fengtai District, Beijing 100071, China*
[b] *Department of Transfusion in Beijing 307 Hospital, Fengtai District, Beijing 100071, China*

Received for publication 15 October 2007; revised 27 November 2007; accepted 7 December 2007

Abstract

High consumption of ethanolic beverages facilitates neurodegeneration, but the mechanism of this process still remained elusive. Suppression subtractive hybridization (SSH) is a technique for detection of rare transcripts. With SSH approach, we identified one ethanol response gene *Cab45*, which was down-regulated by ethanol with time-dependent manner in B104 cells. The full-length sequence of *Cab45* gene was obtained by 5′-RACE (5′ Rapid Amplification of cDNA Ends) for the first time in rat. Based on the sequence of deduced amino acid of rat Cab45, the alignment was conducted with its counterparts in different species and displayed a high conservation. Using different tissues in rat and cell lines, *Cab45* was characterized by a ubiquitous expression and differentiation dependent down-regulation. Given that ethanol facilitates some cell differentiation, we hypothesize that Cab45 is involved in ethanol-mediated differentiation. With transient transfection, the function of Cab45 was investigated by up-regulation and down-regulation in PC12 cells. Ethanol treatment and UV exposure were conducted subsequently and cell proliferations were detected by MTT (Methyl Thiazolyl Tetrazolium) approach. It revealed that the up-regulation of *Cab45* modulated the impairment elicited by ethanol and UV in transfected cells. As a member of new calcium binding protein family, the exact role of Cab45 still remains unclear.

Keywords: ethanol; Cab45; calcium-binding protein; gene identification; subtractive hybridization

Introduction

As a major human disease risk factor, ethanol is involved in many diseases concerned with energy and substrate metabolism, e.g., arteriosclerosis, dementia, diabetes, and conditions associated with Zn metabolism (Kim et al., 2004; Suter, 2004). High consumption of ethanolic beverages increases the risk of certain cancers (Doll and Peto, 1981; Mufti, 1993; Tønnesen et al., 1994) and facilitates neurodegeneration (Fein and Sclafani, 2004; Luchsinger et al., 2004). It has been reported that ethanol exposure leads to impairments of the cerebra and cerebellum in different areas, such as agranular insular cortex, anterior piriform cortex, perirhinal cortex, lateral entorhinal cortex, and the temporal dentate gyrus, and triggers widespread apoptotic neurodegeneration in infant rat and mouse brain (Young et al., 2003). The cause of neurodegeneration by ethanol remains to be elusive and considerable research attention has been focused on identifying the endogenous and environmental factors that contribute to its etiology.

The positive association between ethanol consumption and neurodegeneration was highlighted by recent studies: 1) ethanol induces apoptotic neurodegeneration during the synaptogenesis period of development in rodents (Olney et al., 2002); 2) ethanol selectively decreases Purkinje cell expression of tyrosine-kinase B (TrkB) and tyrosine-kinase C (TrkC) receptors following exposures within postnatal days 4–6. These results suggest that ethanol may induce loss of Purkinje cells by alteration of neurotrophic regulation at this critical stage (Light et al., 2002); 3) using a dual mechanism, which is blockade of N-methyl-D-aspartate (NMDA) glutamate receptors and excessive activation of GABA (A) receptors, ethanol can trigger widespread

* Corresponding author. Fax: +86-10-5112 8448.
E-mail address: zhuyf2004@163.com

apoptotic neurodegeneration in the developing rat forebrain. In humans, vulnerability coincides with the period of synaptogenesis and extends from the sixth month of gestation to several years after birth (Ikonomidou et al., 2000); 4) by suppressing neuronal activity through altering glutamate and GABA transmission, ethanol causes millions of nerve cells to commit death in the developing rodent brain. This pro-apoptotic effect of ethanol provides a likely explanation for the diminished brain size and lifelong neurobehavioral disturbances associated with the human fetal ethanol syndrome (Farber and Olney, 2003)

Based on the phenomenon of ethanol involvement in the process of neurodegeneration, we tried to identify ethanol response genes using suppressed subtractive hybridization (SSH) method. This method offers two major advantages, i.e., the inclusion of all the sequences in the cDNA pool, and the equalization of rare and abundant messages following the initial hybridization (Duguid and Dinauer, 1990). With this method, we identified an ethanol response gene, the counterpart of the mouse and human *Cab45* gene in rat. The time course of expression exhibits a time-dependent down-regulation by ethanol treatment. Based on the fact that ethanol can induce cell differentiation, we investigated *Cab45* expression using cell model of NGF (Neuron Growth Factor)-induced PC12 cells and rat cerebellums with different postnatal days. With the stratagem of up-regulation and down-regulation, *Cab45* was demonstrated to modulate the impairment elicited by ethanol or UV in PC12 cells.

Materials and methods

Cell culture and treatment

B104 cells were routinely grown in RPMI1640 supplemented with 2 mmol/L L-glutamine, 4.5 g/L glucose, 10 mmol/L HEPES, 1 mmol/L sodium pyruvate, 1.5 g/L sodium bicarbonate, 7.2 mg/L insulin, 100 μg/mL Penicillin/Strepmycin, and 10% fetal bovine serum. Cultures were incubated at 37°C with a saturating humidity and 5% CO_2 atmosphere. Medium was changed every 3 days.

Ethanol exposure for B104 Cells

Given to the volatility of ethanol, the sealed containers were used for maintaining ethanol concentration in medium (Adickes et al., 1988; Luo and Miller, 1996). Briefly, the amount of ethanol was calculated in terms of the volume of medium in dish and of water-bath in container (e.g. 0.2%), respectively. A small volume of CO_2 was injected into the container before sealing. The ethanol-containing water bath was changed daily to maintain the ethanol concentration. Cultures were maintained at 37°C in a saturating humidity and 5% CO_2 atmosphere.

Neuron growth factor (NGF) treatment for PC12

PC12 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% horse serum and 10% fetal calf serum (Hyclone, South Logan, UT, USA) (Jean et al., 2005). For experiments, the PC12 cells were incubated with serum-free medium (Dulbecco's modified Eagle's medium containing 2 mg/mL bovine serum albumin, 1 μg/mL insulin, 2 μg/mL transferrin, 30 nmol/L Na_2SO_3, and 10 mmol/L HEPES, pH 7.4) beforehand for 12 h and followed by treatment with NGF (30 ng/mL). The differentiation of PC12 cells induced by NGF was evaluated by synapse outgrowth.

Suppressing subtractive hybridization

Using a commercial kit (Clontech Laboratories, Palo Alto, CA, USA), the subtractive hybridization was performed in B104 cells (rat neuroblastoma cell). Briefly, B104 cells were treated with ethanol (0.2%) for 6 days. Total RNAs derived from both ethanol treated B104 and control were isolated by Trizol regent. mRNAs were prepared by polyA tract kit (Promega, Madison, WI, USA) followed by reverse transcription. cDNAs were digested by *Rsa* I and in which tester cDNA was then ligated with the adaptors provided by the kit. Hybridization was conducted between the tester and the driver, followed by nest PCR amplification with the specific primers provided by the kit (primer1 for the first amplification and nested primer 1 and 2R for the second amplification). PCR products were ligated with the TA vectors pCR4-TOPO (Invitrogen, Carlsbad, CA, USA) to generate recombinant plasmid library (pCR4/SSH). After being transformed into TOP10 cells (Invitrogen, Carlsbad, CA, USA), the clones were cultured for further identification.

Reverse dot blot hybridization

Filters preparation

The transformed clones were picked up randomly, seeded in 96-well plates filled with 100 μL LB broth (containing 50 μg/mL penicillin) per well beforehand, and cultured at 37ºC for 6 h. PCR amplification was conducted for each clone with the primers of Nested primer 1 and 2R and condition was carried out at 95°C for 4 min followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min. PCR products were denatured in 0.6 mol/L NaOH containing 0.5% bromophenol blue and then transferred onto Nytran membranes by Minifold I dot-blotting apparatus (Schleicher & Schuell, Keene, New Hampshire, Germany). Two filters were prepared for each pattern.

Labeled cDNA library preparation

Both labeled cDNA libraries (driver and tester) were

Download English Version:

https://daneshyari.com/en/article/2788017

Download Persian Version:

https://daneshyari.com/article/2788017

Daneshyari.com