



Research Article / Araştırma Makalesi

DIFFERENTIAL EXPRESSION OF BDNF, MIR-206 AND MIR-9 UNDER THE CHRONIC ETHANOL EXPOSURE AND ITS WITHDRAWAL

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ABSTRACT

Chronic and acute alcohol abuse results in alters in the expression of some miRNAs and their mRNA targets in specific area of the brain. These expression changes might cause the cellular adjustments to long term alcohol abuse. We searched BDNF, miR-9 and miR-206 expression changed in SH SY5Y cell following exposure to ethanol and withdrawal. These miRNAs and BDNF are estimated to target genes of alcoholism. Chronic ethanol exposure and its removal showed that specific changes in miRNA and BDNF expression in cell line advocating that different expressions could be evoked with different exposure conditions. Especially, there were three group in our experiment one of them 10 days Chronic ethanol exposure, other one 5 days chronic ethanol exposure then 5 days normal medium without ethanol for withdrawal period and other group was control. After all, miR-9 was up-regulated after ethanol treatment but was downregulated in withdrawal condition. In addition miR-206 was up-regulated all condition. Whereas BDNF gene expression was regulated opposite direction according to miRNA expression. Our results suggest that a possible role of differentially expressed miRNAs and BDNF perform together in mediating alcohol influence.

Keywords: BDNF, miRNA, ethanol exposure, SHSY5.

KRONİK ALKOL UYGULANMASI VE YOKSUNLUĞUNDA BDNF, MIR-206 ve MIR-9' un EKSPRESYON DEĞİŞİMİ

ÖZ

Kronik ve akut alkol kullanımı beynin bazı bölgelerindeki mRNA ve miRNA ekspresyonunu değiştirmektedir. Bu değişimler hücrel değişimlere de sebep olabilmektedir. Bu çalışmada alkol muamelesi ve yoksunluğunda SH SY5Y hücreleri üzerinde BDNF, miR-206 ve miR-9 ekspresyon değişimini araştırdık. Bu miRNAların alkolizm durumunda BDNF ile ilişkili olduğu tahmin edilmektedir. Hücrelerimizi 3 gruba ayırdık. Bunlardan bir geuba 10 gün boyunca belirlenen konsantrasyonda etanol verildi. Diğer gruba 5 gün etanol verilip yoksunluk oluşturmak için 5 gün normal medyum verildi. Son grup da kontrol grubu olarak 10 gün boyunca sadece normal medyum verildi. Sonuçta miR-9' un alkolik grupta ekspresyonu arttığı ancak yoksunlukta ekspresyonu azaldığı görüldü. miR-206' nın ekspresyonu her koşulda artmıştır. BDNF geninin ekspresyonu da miRNA ekspresyonuna göre ters yönde değişim göstermiştir. Buna göre alkol bağımlılığında ve yoksunluğunda mRNA ve miRNA birlikte çalıştığı gösterilmiştir.

Anahtar Sözcükler: BDNF, miRNA, etanol uygulaması, SHSY5Y.

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1. INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is a neurotrophic component has shown in the nervous system, with greatest expression in the hippocampus and cortex [1,2]. BDNF signaling is regarded to be incorporated in synaptic remodeling [2,3] synaptic plasticity [4], learning and memory [5-7], and addiction [8]. Surprisingly, BDNF has been linked to pathways that negative relationship the ethanol, and some evidence from human researches reported that genetic variations which fell the purpose of the BDNF pathway are prevalent in alcoholics than in the overall population. All of these data claimed that BDNF role arrest the alcoholism improvement. Some proofs additionally demonstrates that the role of BDNF display negative relationship between ethanol using rats. Additionally, rat strains susceptible to ingest higher amounts of ethanol demonstrated decreased BDNF expression when contrasted to their reduced-ingesting ones [9-12]. Current researches have displayed that the medial prefrontal cortex (mPFC) is fragile to durable modifications in gene expression after alcohol dependence [13-15,11]. Newly, some microarray researches were showed to classify dysregulated microRNA expression in the medial prefrontal cortex in rats [16,15,11]. One of the differentially expressed miRNAs, miR-206 was upregulated following alcoholism. miR-206 is significant due to the fact that repressed the BDNF [17,18,15,11]. Various behavioral results have been seen to BDNF for alcohol. We discovered different expression pattern of miR-206 and miR-9 and their target BDNF in the SHSY5Y cell line after alcohol exposure and withdrawal. These results suggest miR-206 directly regulate to BDNF but miR-9 indirectly regulate to BDNF. Also this results and cell line model can be used to clarify the role of miRNAs-mediated gene regulation in alcohol exposure and withdrawal.

2. MATERIALS-METHODS

2.1. Cell culture and Cytotoxicity

SH-SY5Y cells were used for cytotoxicity and ethanol exposure studies. Cells were cultured in DMEM-F12 with 10% FBS at 37°C, 5% CO₂. The medium of culture was changed once in 2 days. Cell cytotoxicity was measured by the MTT assay according to manufacturer's protocol (Sigma USA) [19,20]. Cells were plated as 10³ cells per well in 96 well plates. After 24 h of incubation, ethanol was added at different concentrations. After 48 h, 20 µL MTT solution (5 mg/mL) was added into each well. After additional incubation for 4 h, 100 µL DMSO was added. The absorbance values were measured at 495 nm with a microplate reader (Biotek synergy, USA).

2.2. Ethanol Exposure

The cells were seeded into six well plates appropriate concentration then were divided to three groups. Control group that was used just normal media without ethanol for 10 days. Chronic ethanol group was exposed to 75 mM ethanol continuously for 10 days. Withdrawal group was exposed 75 mM ethanol continuously for 5 days and following 5 days exposed to without ethanol media.

2.3. RNA Isolation

Total RNA was isolated with Trizol reagent according to the manufacturer's instructions (Invitrogen Life Technologies, USA). miRNAs were isolated from each samples using the miRNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions and total RNA concentration was measured on the NanoDrop (ThermoScientific, USA).

2.4. q-RT PCR and Data analysis

From the each samples 100 ng total isolated RNA was reverse transcribed using “RevertAid™ First Strand cDNA Synthesis” kit (Thermo scientific, USA) following the manufacturer’s instructions. Samples were assayed in triplicate in a total reaction volume of 15 ml using SYBR Green based All-in-One™ qPCR Mix(Genecopoeia, USA) on BioRAD CFX 96 RT-PCR system(40 cycle of 95 C for 10 min initial denaturation, 95 C for 10 sec for denaturation, 58 C for 20 sec for annealing and 72 C for 20 sec for extension steps.) Using primers for BDNF, miR-206 and miR-9 were designed according to NCBI primer design programe. BioRad CFX96 analysis software was used for determination of SYBR green fluorescence intensity and to calculate the theoretical cycle number when threshold was passed (Ct values). Expression level determination was done according to $2^{-\Delta\Delta CT}$ metod. (Livak and Schmittgen 2008) . Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as internal control. The $\Delta\Delta Ct$ values were compared by student t-test (P<0.05 significance; P<0.07 trend).

3. RESULT AND DISCUSSIONS

3.1. Methabolic Activity

It was determined the proper ethanol consantration as 75 mM (Figure 1). After 75 mM ethanol was exposed, cell started to die. There were no significant value up to 50 mM concentration of ethanol. This data showed that long term ethanol use cause tissue defect such as organ and brain damage. [21]

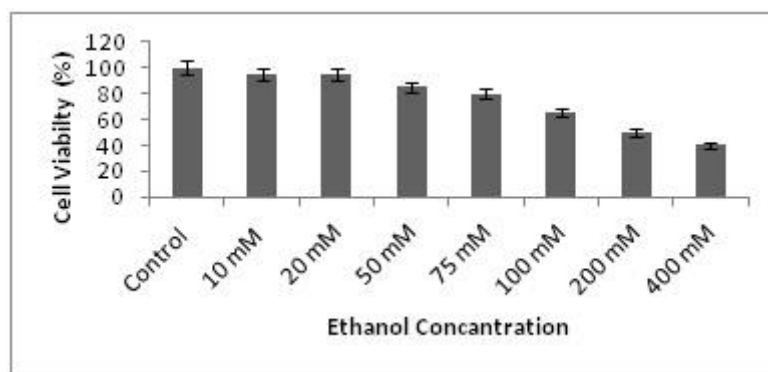


Figure 1. Cytotoxicity under the different ethanol concentrations

3.2. Regulation of BDNF, miR-206 and miR-9

We showed that in ethanol exposure on SHSY5Y cell lines miR-206 expression was increased whereas BDNF expression was decreased(Figure 2). That means miR-206 binds to BDNF 3' UTR and inhibits the expression .

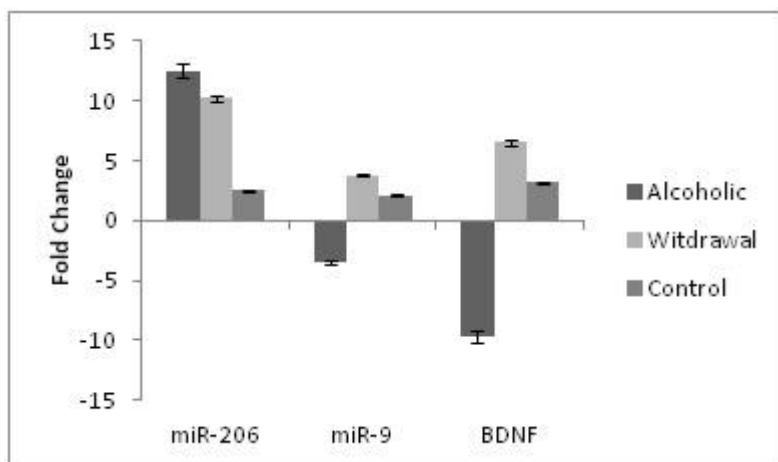


Figure 2. Gene expression changes under the ethanol stress

miR-206 attaches to BDNF 3' UTR and blocks the expression. Tapocik et al. also showed that interaction between miR-206 expression and alcohol-related behaviors, when overexpression of miR-206 in nondependent rats delayed BDNF expression in vivo [15]. This aids the presumption that modifications of miR-206 expression aid to regulation of alcohol own-management by the medial prefrontal cortex and classify BDNF mechanism [15,11]. miR-206 is recognized as a skeletal muscle-specified miRNA that is required for renewal following intense nerve damage [15,11,22]. In the people or rats brain, miR-206 is expressed at quite limited amounts, yet its expression is stimulated below illness circumstances, like in Alzheimer's disease. For example, higher expression of miR-206 was displayed in Alzheimer's disease in brains of transgenic mice and in the temporal cortex of Alzheimer's illness people [17,2,15,11]. BDNF is affected in the regulation of synaptic plasticity, which is regulated under drugs abuse [23,15,11]. In addition, BDNF activation has been shown to trigger mainly three signaling pathways that are mitogen-activated protein kinase (MAPK), phosphatidylinositol-3-kinase (PI3K), and phospholipase-Cg (PLC-g), which can be included in drugs of abuse [24,15,11]. All of them, we demonstrated that miR-206 was upregulated and BDNF was downregulated in ethanol addiction. On the other hands miR-9 is important to relevant to ethanol concentration and increase in central nervous systems. Acute ethanol process was discovered to trigger a quick improve in miR-9 expression in cultured neurons [25] but we demonstrated that in ethanol exposure miR-9 expression was downregulated whereas withdrawal condition it was upregulated. The miR-9 reliant on downregulation of desired mRNA may demonstrate a common mechanism of neuronal adjustment to alcohol. These adaptations are neuronal physiology incorporating excitability, gene expression's regulation, lipids metabolism and presynaptic terminals function.

4. CONCLUSIONS

We try to determine effect of ethanol exposure on SHSY5Y cell lines like brain cells. Our results suggest that BDNF and its target miR-206 are very important in alcohol addiction whereas miR-9 is not related to BDNF gene and its expression. miR-9 and BDNF gene show different expression pattern so they don't work together. In contrast, BDNF and miR-206 work together and regulate gene expression under the alcohol addiction. That is mean BDNF and miR-206 are proper therapeutic agent for addiction.

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