Sigma J Eng & Nat Sci 34 (2), 2016, 191-196

Sigma Journal Engineering and Natural Sciences
Sigma Mühendislik ve Fen Bilimleri Dergisi

Research Article / Araştırma Makalesi

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

Zeynep AYDIN SİNİRLİOĞLU*[1], Fahri AKBAŞ[2]

[1]*Department of Genetics and Bioengineering, Fatih University, Buyukcekmece-ISTANBUL*
[2]*Medical Biology Department, Bezmialem University, Fatih-ISTANBUL*

Received/Geliş: 08.04.2016 Revised/Düzeltme: 10.05.2016 Accepted/Kabul: 18.05.2016

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 adjusments 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 hücresel 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 alkolizim durumnunda 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 grupda 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.

* Corresponding Author/Sorumlu Yazar: e-mail/e-ileti: zeynepaydin@fatih.edu.tr, tel: (212) 866 33 00 / 5619

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 37oC, 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 10^3 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 approprite 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 anneling 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 ΔΔCt 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 exposured, 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]

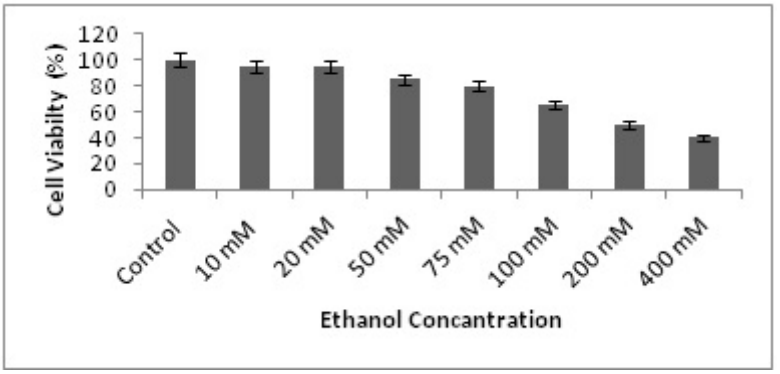

Figure 1. Cytotoxity under the different ethanol concentrations

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 .

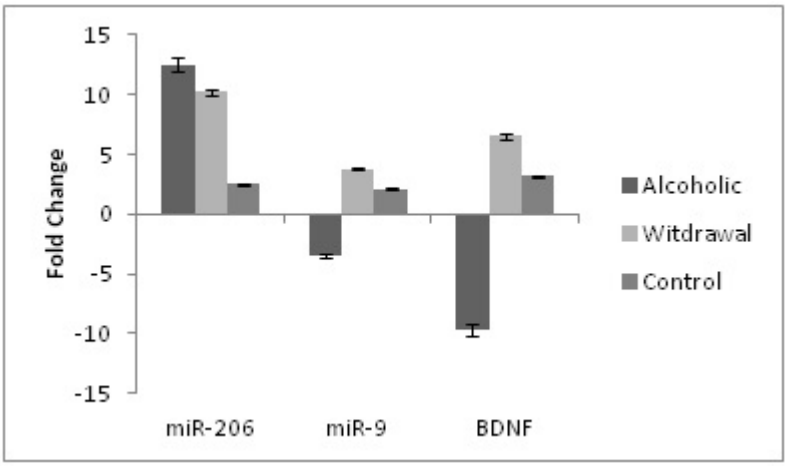

Figure 2. Gene expression changes under the ethanol stress

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 corteks 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 demonsrated 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 alcahol addiction whereas miR- 9 is not releated 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 therapatic agent for addiction.

Acknowledgments / Teşekkür

This study was supported by Fatih University Research Project Foundation (P50031401_B).

REFERENCES / KAYNAKLAR

1] Hofer M, Pagliusi SR, Hohn A, Leibrock J, Barde Y (1990) Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. The EMBO journal 9 (8):2459.

[2] Logrip ML, Janak PH, Ron D (2009) Escalating ethanol intake is associated with altered corticostriatal BDNF expression. Journal of neurochemistry 109 (5):1459-1468.

[3] Lu B (2003) BDNF and activity-dependent synaptic modulation. Learning & memory 10 (2):86-98.

[4] Bramham CR, Messaoudi E (2005) BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. Progress in neurobiology 76 (2):99-125.

[5] Tyler WJ, Alonso M, Bramham CR, Pozzo-Miller LD (2002) From acquisition to consolidation: on the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. Learning & memory 9 (5):224-237.

[6] Yamada K, Mizuno M, Nabeshima T (2002) Role for brain-derived neurotrophic factor in learning and memory. Life sciences 70 (7):735-744.

[7] Yamada MK, Nakanishi K, Ohba S, Nakamura T, Ikegaya Y, Nishiyama N, Matsuki N (2002) Brain-derived neurotrophic factor promotes the maturation of GABAergic mechanisms in cultured hippocampal neurons. The Journal of Neuroscience 22 (17):7580-7585.

[8] Russo SJ, Mazei-Robison MS, Ables JL, Nestler EJ (2009) Neurotrophic factors and structural plasticity in addiction. Neuropharmacology 56:73-82.

[9] Pandey SC, Zhang H, Roy A, Misra K (2006) Central and medial amygdaloid brain-derived neurotrophic factor signaling plays a critical role in alcohol-drinking and anxiety-like behaviors. The Journal of Neuroscience 26 (32):8320-8331.

[10] Prakash A, Zhang H, Pandey SC (2008) Innate Differences in the Expression of Brain-Derived Neurotrophic Factor in the Regions Within the Extended Amygdala Between Alcohol Preferring and Nonpreferring Rats. Alcoholism: Clinical and Experimental Research 32 (6):909-920.

[11] Tapocik JD, Solomon M, Flanigan M, Meinhardt M, Barbier E, Schank J, Schwandt M, Sommer WH, Heilig M (2013) Coordinated dysregulation of mRNAs and microRNAs in the rat medial prefrontal cortex following a history of alcohol dependence. The pharmacogenomics journal 13 (3):286-296.

[12] Yan Q-S, Feng M-J, Yan S-E (2005) Different expression of brain-derived neurotrophic factor in the nucleus accumbens of alcohol-preferring (P) and-nonpreferring (NP) rats. Brain research 1035 (2):215-218.

[13] Meinhardt MW, Hansson AC, Perreau-Lenz S, Bauder-Wenz C, Stählin O, Heilig M, Harper C, Drescher KU, Spanagel R, Sommer WH (2013) Rescue of infralimbic mGluR2 deficit restores control over drug-seeking behavior in alcohol dependence. The Journal of Neuroscience 33 (7):2794-2806.

[14] Melendez RI, McGinty JF, Kalivas PW, Becker HC (2012) Brain region-specific gene expression changes after chronic intermittent ethanol exposure and early withdrawal in C57BL/6J mice. Addiction biology 17 (2):351-364.

[15] Tapocik JD, Barbier E, Flanigan M, Solomon M, Pincus A, Pilling A, Sun H, Schank JR, King C, Heilig M (2014) microRNA-206 in rat medial prefrontal cortex regulates BDNF expression and alcohol drinking. The Journal of Neuroscience 34 (13):4581-4588.

[16] Lewohl JM, Nunez YO, Dodd PR, Tiwari GR, Harris RA, Mayfield RD (2011) Up-Regulation of MicroRNAs in Brain of Human Alcoholics. Alcoholism: Clinical and Experimental Research 35 (11):1928-1937.

[17] Lee ST, Chu K, Jung KH, Kim JH, Huh JY, Yoon H, Park DK, Lim JY, Kim JM, Jeon D (2012) miR-206 regulates brain-derived neurotrophic factor in Alzheimer disease model. Annals of neurology 72 (2):269-277.

[18] Miura P, Amirouche A, Clow C, Bélanger G, Jasmin BJ (2012) Brain-derived neurotrophic factor expression is repressed during myogenic differentiation by miR-206. Journal of neurochemistry 120 (2):230-238.

[19] Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB (1987) Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. Cancer research 47 (4):936-942.

[20] Yücel S, Aydın Sinirlioğlu Z, Karakuzu B, Temel TM, Elalmış Y, Özarslan AC Production, Characterization and Bioactivity of 46S6 Bioactive Glass from Rice Hull Ash Silica. In: Advanced Materials Research, 2015. Trans Tech Publ, pp 857-861.

[21] Thibault C, Lai C, Wilke N, Duong B, Olive MF, Rahman S, Dong H, Hodge CW, Lockhart DJ, Miles MF (2000) Expression profiling of neural cells reveals specific patterns of ethanol-responsive gene expression. Molecular Pharmacology 58 (6):1593-1600.

[22] Williams AH, Valdez G, Moresi V, Qi X, McAnally J, Elliott JL, Bassel-Duby R, Sanes JR, Olson EN (2009) MicroRNA-206 delays ALS progression and promotes regeneration of neuromuscular synapses in mice. Science 326 (5959):1549-1554.

[23] Sadri-Vakili G, Kumaresan V, Schmidt HD, Famous KR, Chawla P, Vassoler FM, Overland RP, Xia E, Bass CE, Terwilliger EF (2010) Cocaine-induced chromatin remodeling increases brain-derived neurotrophic factor transcription in the rat medial prefrontal cortex, which alters the reinforcing efficacy of cocaine. The Journal of Neuroscience 30 (35):11735-11744.

[24] Nagappan G, Lu B (2005) Activity-dependent modulation of the BDNF receptor TrkB: mechanisms and implications. Trends in neurosciences 28 (9):464-471.

[25] Pietrzykowski AZ, Friesen RM, Martin GE, Puig SI, Nowak CL, Wynne PM, Siegelmann HT, Treistman SN (2008) Posttranscriptional regulation of BK channel splice variant stability by miR-9 underlies neuroadaptation to alcohol. Neuron 59 (2):274-287.