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Construction and Transfection of EGFP-N1-BDNF Expression Plasmid

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Abstract: Brain-Derived Neurotrophic Factor (BDNF) is a member of the neurotrophin family of growth factors and plays significant role in central nervous system and the peripheral nervous system for supporting the survival of existing neurons and encouraging the growth and differentiation of new neurons and synapses. Increasing study indicate that BDNF is associated with neurological disorders and psychiatric diseases. Beyond the importance of BDNF in nervous system, compelling evidence also demonstrate that it is also essential for body weight control and energy homeostasis. BDNF therefore, become the target in various diseases research such as Alzheimer's disease, depressive disorder and obesity. In this study, researchers cloned the *BDNF* gene to p-EGFP N1 expression plasmid, meanwhile, researchers also transfected the p-EGFP N1-BDNF to Hela cell with lipofection. Fluorescence microscope and Western blot analysis showed BDNF could normally express in Hela cell. However, the physiological functions and pharmacological roles of the p-EGFP N1-BDNF need further validation in cellular level and animal model.

Key words: Brain-derived neurotrophic factor, Alzheimer's disease, obesity, lipofection, China

INTRODUCTION

Along with Nerve Growth Factor (NGF) and Neurotrophin (NT) 3, NT 4/5 and NT 6, Brain-Derived Neurotrophic Factor (BDNF) is a member of the neurotrophin family of growth factors. BDNF plays significant role in Central Nervous System (CNS) and the Peripheral Nervous System (PNS), exerting to support the survival of existing neurons and encourage the growth and differentiation of new neurons and synapses (Huang and Reichardt, 2001). Meanwhile, it is active in the hippocampus, cortex and basal forebrain-areas which are vital to learning, memory and higher cognitive functions (Yamada and Nabeshima, 2003) (Bekinschtein *et al*., 2008). Increasing study indicate that BDNF exercises its multitude roles such as the promotion of development and differentiation of neurons, cell survival, long-term potentiation and synaptic plasticity mainly through its interaction with the Tropomyosin-related kinase B (TrkB) (Patapoutian and Reichardt, 2001; Poo, 2001). TrkB, also named Neurotrophic Tyrosine Kinase Receptor type 2 (NTRK2) is first identified as a highly expressed protein-tyrosine kinase in the brain and together with two other members, TrkA and TrkC, comprises the Tyrosine kinase (Trk) family of neurotrophin receptors (Huang and Reichardt, 2001).

Upon ligand binding by BDNF, the full-length TrkB forms homodimers leading to the autophosphorylation of tyrosine residues in the cytoplasmic domains which results in the activation of downstream signaling pathways (Huang and Reichardt, 2001; Reichardt, 2006). The downstream pathways activated by TrkB signaling include Mitogen-Activated Protein Kinase (MAPK), PhospholipaseCc (PLC-c), Phosphatidylinositol-3-Kinase (PI3K) and Protein Kinase C (PKC) cascades (Fig. 1) (Huang and Reichardt, 2001; Fenner, 2012). As BDNF plays a crucial role in development and plasticity of the brain, Gene mutations and abnormal protein expression of BDNF are associated with neurological disorders such as Alzheimer's disease, Parkinson's disease and Huntington's disease and is widely implicated in psychiatric diseases including depressive disorder, schizophrenia, addiction, Rett syndrome and post traumatic stress disorder (Murer *et al*., 2001; Zuccato *et al*., 2008; Buckley *et al*., 2011; Pivac *et al*., 2011; Autry and Monteggia, 2012).

Based on the multiple functions of BDNF in CNS and PNS, it has been targeted widely to treat various neurological and psychiatric diseases. In current study, researchers also cloned the *BDNF* gene to p-EGFP N1 expression plasmid, meanwhile researchers also transfected the p-EGFP-BDNF to Hela cell with

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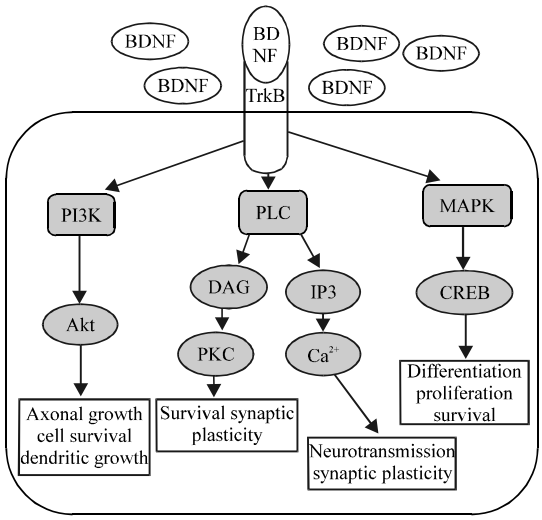

Fig. 1: BDNF exercises its multitude roles through its interactions with the TrkB (Huang and Reichardt, 2001; Fenner, 2012)). Upon ligand binding by BDNF, TrkB forms homodimers leading to the autophosphorylation of tyrosine residues in the cytoplasmic domains which results in the activation of downstream signaling pathways include MAPK, PLC and PI3K. BDNF: Brain-Derived Neurotrophic Factor, TrkB: Tropomyosin-related kinase B, MAPK: Mitogen-Activated Protein Kinase, PLC: PhospholipaseC, PI3K: Phosphatidylinositol-3-Kinase, DAG: Diacylglycerol; IP3: Inositol triphosphate, PKC: Protein Kinase C, Ca^{2+}: Calcium ion; CREB: cAMP response element binding; Akt: Protein kinase B

lipofection. Furthermore, the results of transfection were analyzed by fluorescence microscope and Western blot analysis.

MATERIALS AND METHODS

Cell line: The human Hela cell line was obtained from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences and cultured in DMEM containing 10% fetal bovine serum, at 37°C with 5% volume per volume CO_2.

p-EGFP-N1-BDNF construction: Human testis cDNA was generously provided by Dr. Tan from Chinese Academy of Sciences. The 742 bp of BDNF was amplified by PCR and cloned into PuCM-T simple vector which is an Invitrogen product, USA. The PuCM-T-BDNF vector was digested by restriction endonucleases Hind III and Sal I to generate a ~742 bp Hind III-BDNF-Sal I fragment. This fragment was then ligated into identical sites contained in p-EGFP N1 plasmid (Clontech, Palo Alto, CA) to synthesize the p-EGFP-N1-BDNF plasmid. The sequences of the inserts were checked by restriction enzyme digestion and the DNA sequencing.

Transfection: The p-EGFP-N1-BDNF vector or p-EGFP empty vector was transfected into Hela cells by Lipofectamin 2000 (Invitrogen, USA) according to the manufacture's instruction. The transfected cells were analyzed by fluorescence microscope at 48 h post transfection.

Protein extraction and Western blot analysis: Protein levels were extracted from cells transfected with p-EGFP-BDNF or p-EGFP for 48 h and quantified by Bradford assay. Total of 50 μg protein from each sample were fractionated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto nitrocellulose membrane (BioRad Laboratories, Richmond, CA). The membrane was blocked in 5% nonfat dry milk for 1 h at 4°C and then probed with anti-BDNF (1:5000, Abcam, Cambridge, MA) mouse monoclonal primary antibodies. After washing 3 times with Tris-buffered saline Tween-20, the membrane was incubated in alkaline phosphatase-labeled secondary antibody (1:1000, Santa Cruz Biotechnology). Bands were visualized by nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate solution and quantified by ImageJ Software (NIH, Bethesda, MD).

RESULTS AND DISCUSSION

Establishment of stable transfectants: p-EGFP-BDNF and p-EGFP control vectors were transfected into Hela cells. At 48 h after transfection, fluorescent microscopy showed GFP-t and GFP-BDNF cells emitted green fluorescence (Fig. 2). Meanwhile, Western bolt analysis indicated that BDNF expressed in the Hella cells (Fig. 3). The study has important implications on the preventive medicine research for increasing study indicates that BDNF is associated with neurological disorders and psychiatric diseases.

Besides that this neurotrophic factor has major regulatory role in the control of food intake, body weight and metabolism thus plays a major role in certain types of obesity even eating disorders such as Anorexia Nervosa (AN) and Bulimia Nervosa (BN) (Nakazato *et al*., 2012). Increasing papers support this compelling idea. For example, low levels of circulating BDNF have been found in individuals who suffer from obesity and type 2 diabetes (Krabbe *et al*., 2007) and the inverse association between

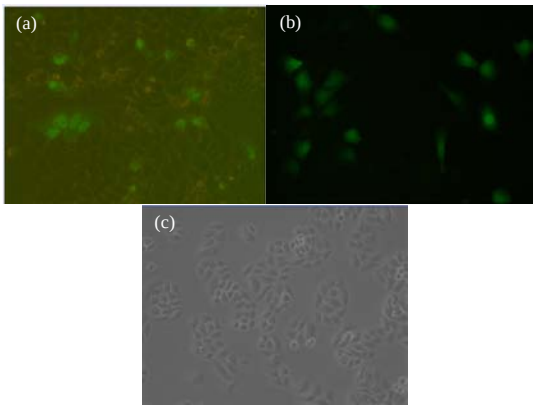

Fig. 2: The transfection was analyzed by fluorescence microscope at 48 h post transfection. a) Helle cells transfected with the p-EGFP-N1-BDNF vector. b) Hella cells transfected with the p-EGFP empty vector. c) Hella cells with no treatment

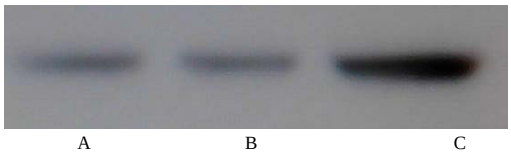

Fig. 3: Western bolt analyze BDNF expression in the Hela cells. A) Hella cells with no treatment. B) Hella cells transfected with the p-EGFP empty vector. C) Hella cells transfected with the p-EGFP-N1-BDNF vector

the peripheral BDNF concentration and Body Mass Index (BMI) is found in children and adults (Lommatzsch *et al*., 2005). Besides that a child with low levels of serum BDNF exhibits severe obesity and hyperphagia as well as a complex neurobehavioral phenotype (Gray *et al*., 2006). Also, one more detailed case, a hyperphagic obese child is described with a heterozygous missense substitution resulting in impaired signaling of the cognate receptor of BDNF, TrkB (Yeo *et al*., 2004). Obesity, refereed as an excessive accumulation of body fat is a serious health problem whose incidence is on the rise in developed and developing countries. According to the global estimation of World Health Organization (WHO) from 2008, about 1.5 billion adults are overweight. Of these, over 200 million men and nearly 300 million women are obese. Equally perturbing are the 43 million children less 5 years of age who are overweight in 2010 (De Onis *et al*., 2010; Rosas-Vargas *et al*., 2011). This finding therefore is very important and has significant implications on human health because it represents a potential target for developing new therapies for obesity. In fact, study have found that the central and peripheral administration of BDNF decrease food intake, increase energy expenditure and ameliorate hyperinsulinemia and hyperglycemia by a central nervous system-mediated mechanism in animal models (Nakagawa *et al*., 2000; Nonomura *et al*., 2001; Xu *et al*., 2003).

CONCLUSION

In current study, researchers cloned the *BDNF* gene to p-EGFP N1 expression plasmid, meanwhile, researchers also transfected the p-EGFP-BDNF to Hela cell with lipofection. Fluorescence microscope and Western blot analysis showed BDNF could normally express in Hela cell. However, the physiological function and pharmacological role need further validation in cellular level and animal model.

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