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Expression and regulation of antiviral protein APOBEC3G in human neuronal cells ☆

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ABSTRACT

Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G (APOBEC3G) has recently been identified as a potent antiviral protein. Here, we examined the expression and regulation of APOBEC3G in human brain tissues and the cells of central nervous system (CNS). Similar to the immune cells, human brain tissue and the CNS cells expressed APOBEC3G at both mRNA and protein levels. The expression of APOBEC3G could be up-regulated in human neuronal cells (NT2-N) and astrocytes (U87-MG) by interferons (IFN-α, β and γ), interleukin-1 (IL-1), and tumor necrosis factor. Other cytokines (IL-4, IL-6 and transforming growth factor beta1) and CC-chemokines (CCL3, 4 and 5), however, had little impact on the expression of APOBEC3G. In addition, pseudotyped HIV-1 infection and cytokine/chemokine-enriched supernatants from lipopolysaccharide-stimulated macrophage cultures induced APOBEC3G expression in NT2-N cells. APOBEC3G expressed in the neuronal cells and astrocytes was biologically functional, as the suppression of APOBEC3G expression by the specific siRNA led to increase of pseudotyped HIV-1 replication in these cells. These findings provide direct and compelling evidence that there is intracellular expression and regulation of functional APOBEC3G in the neuronal cells, which may be one of innate defense mechanisms involved in the neuronal protection in the CNS.

1. Introduction

APOBEC3G, a member of the apolipoprotein B mRNA-editing enzyme catalytic polypeptide (APOBEC) family of proteins homologous cytidine deaminase domains (Jarmuz et al., 2002), has been identified as a host factor involved in cellular defense mechanism against retroviruses (Harris et al., 2003; Mangeat et al., 2003; Sheehy et al., 2002). APOBEC3G can either edit the newly synthesized viral DNA or inhibit another site(s) of the viral life cycle (Mangeat et al., 2003; Mariani et al., 2003; Turelli and Trono, 2005; Zhang et al., 2003). Recently, inhibition of human immunodeficiency virus type 1 (HIV-1), simian immunodeficiency virus, equine infectious anemia virus, murine leukemia virus and hepatitis B virus by APOBEC3G has been reported (Mangeat et al., 2003; Navarro and Landau, 2004; Noguchi et al., 2005; Rosler et al., 2005).

Although APOBEC3G resides in HIV-1 permissive cells, such as $CD4^+$ T cells and monocyte-derived macrophages (Chiu et al., 2005), expression of APOBEC3G has also been identified in other human organs, such as lungs, testes, ovaries, liver and kidney (Chen et al., 2006; Jin et al., 2005; Komohara et al., 2007, 2006; Turelli and Trono, 2005). In addition, APOBEC3G could be induced by interferons (IFNs) in human lymphocytes, macrophage, liver and kidney cells (Bonvin et al., 2006; Chen et al., 2006; Komohara et al., 2007, 2006; Peng et al., 2006; Tanaka et al., 2006). Both type I and type II IFNs play a critical role in the host innate immunity against viral infections. IFN-α is a potent inhibitor of HIV-1 infection of $CCR5^+$ $CD4^+$ human macrophages (Baca-Regen et al., 1994; Meylan et al., 1993). IFN-α is also a potent inducer of APOBEC3G, which overrides HIV-1 vif-mediated neutralization of APOBEC3G protein that pose a threat to efficient HIV-1 replication in macrophages (Peng et al., 2006). Although it has been demonstrated that APOBEC3G could be induced by type I IFNs in human lymphocytes,

Abbreviation: APOBEC3G, apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G; BDNF, brain-derived neurotrophic factor; BMVECs, brain microvascular endothelial cells; CCL3, CC chemokine ligand 3; CCL4, CC chemokine ligand 4; CCL5, CC chemokine ligand 5; CNS, central neurons system; DMEM, Dulbecco's modified Eagle's medium; DSU, densitometry scanning unit; FBS, fetal calf serum; hEGF, human epidermal growth factor; hFGFb, human fibroblast growth factor-basic; HIV-1, human immunodeficiency virus type 1; IFN-α, interferon alpha; IFN-β, interferon beta; IFN-γ, interferon gamma; IL-1β, interleukin-1 beta; IL-4, interleukin-4; IL-6, interleukin-6; IMDM, Iscover's modified Dulbecco's medium; LPS, lipopolysaccharide; NMDA, *N*-methyl-D-aspartate; NPC, neural precursor cell; PBL, peripheral blood lymphocytes; RA, retinoic acid; TGF-β1, transforming growth factor beta1; TNF-α, tumor necrosis factor alpha.

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macrophage, liver and kidney (Bonvin et al., 2006; Chen et al., 2006; Komohara et al., 2007, 2006; Peng et al., 2006; Tanaka et al., 2006), there is limited information about the expression and regulation of APOBEC3G in human central nervous system (CNS), an important target for viral infections, including HIV-1. Therefore, we examined whether the CNS cells express functional APOBEC3G, and whether IFNs (alpha, beta and gamma) and other cytokines/chemokines modulate the expression of APOBEC3G in the human neuronal cells.

2. Materials and methods

2.1. Primary cells

Human brain tissues used were obtained from the National Neurological Research Specimen Bank (Los Angeles, CA). Neural precursor cell (NPC)-derived human neuronal cells were kindly obtained from Dr. Hu, Shuxian (The Center for Infectious Diseases and Microbiology Translational Research, Department of Medicine, University of Minnesota Medical School, Minneapolis, Minnesota). NPC cultures were prepared from the telencephalon of 8- to 10-week-old human fetal brain using previously described methods (Hu et al., 2006, 2002; Ni et al., 2004; Uchida et al., 2000). Peripheral blood samples were obtained from healthy adult donors. The Institutional Review Board of the Children's Hospital of Philadelphia approved this investigation. Informed consent was obtained from the subjects. Peripheral blood lymphocytes (PBL) and monocytes were processed as described previously (Hassan et al., 1986). Freshly isolated monocytes were plated in 48-well culture plates at a density of 0.5×10^6 cells per well in DMEM containing 10% fetal bovine serum (FBS) (Invitrogen). Macrophages referred to 7-day-cultured monocytes. Monocyte/macrophage viability was monitored by trypan blue exclusion and maintenance of cell adherence. In all cases, limulus amebocyte lysate assay demonstrated that media and reagents were endotoxin-free.

2.2. Cell lines

Human neuronal cells (NT2-N) were derived from differentiated Ntera-2clD/1 (NT2) cells as described previously (Andrews, 1984). In brief, NT2 cells were plated at a density of 2.3×10^6 per T75 flask and fed twice weekly with DMEM containing high glucose (Gibco, Grand Island, NY, USA) and 10% FBS (Hyclone, Iogan, UT, USA) with 100 U/ml penicillin plus 100 μg/ml streptomycin (Gibco) and 10^{-5} M retinoic acid (RA) (Sigma-Aldrich, St. Louis, MO, USA) for 5 weeks. The cells were then divided (1:4) and grown for an additional 48 h in identical medium without RA. Neuronal cells growing above a monolayer of non-neuronal cells were dislodged with trypsin and plated at a density of 0.5×10^6 cells per well in a 24-well plate for this study. NT2-N neurons have morphologic features similar to primary human neurons, and have processes that differentiate into axons and dendrites (Andrews, 1984). NT2-N neurons also express cytoskeletal proteins, secretory markers, and surface markers, which are characteristic of neurons. They also express functional neuropeptides (Guillemain et al., 2000) and functional *N*-methyl-D-aspartate (NMDA) and non-NMDA glutamate receptors (Younkin et al., 1993). Undifferentiated human NT2 cells grafted into mouse brain differentiated into neuronal and glial cells (Ferrari et al., 2000).

The neuroblastoma cell lines (CHP212 and SY5Y) were purchased from American Type Tissue Culture (ATCC; Manassas, VA). CHP212 cells were cultured in Eagle's MEM-Ham F12 (1:1) medium containing 10% FCS, 0.1 mM non-essential amino acid and 1.0 mM sodium pyruvate. SY5Y cells were propagated in DMEM with high glucose containing 10% FBS. Human astroglioma cells (U87-MG) and human B lymphoblasts

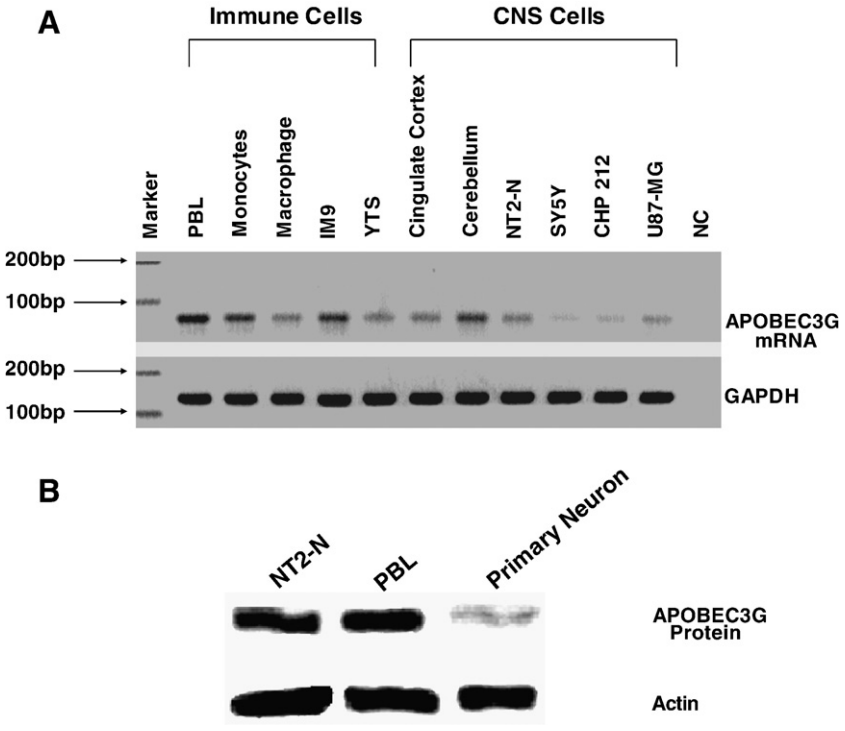

Fig. 1. APOBEC3G expression in the human immune cells, brain tissues and CNS cells. (A) RT-PCR analysis of APOBEC3G mRNA expression. Total cellular RNA extracted from the indicated cells was subjected to the real time RT-PCR using the specific primer pairs for APOBEC3G (70 bp) and GAPDH (127 bp). The amplified (35 cycles) products were then subjected to electrophoresis on 3% ultra pure agarose gel; (B) Western blot analysis of APOBEC3G protein expression. Proteins extracted from NT2-N cells, human peripheral blood lymphocytes (PBL) and primary human neurons (neural precursor cell-derived human neuronal cells) were subjected to Western blot assay using rabbit antibodies against APOPEC3G and actin. One representative of 3 independent experiments with similar results is shown.