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BRAIN RESEARCH

Research Report

Interferon β-1b directly modulates human neural stem/progenitor cell fate

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ARTICLE INFO

Article history:
Accepted 18 July 2011
Available online 23 July 2011

Keywords:
Multiple sclerosis
Interferon-beta
Neural progenitor cells

ABSTRACT

Interferon beta (IFN-β) is a mainline treatment for multiple sclerosis (MS); however its exact mechanism of action is not completely understood. IFN-β is known as an immunomodulator; although recent evidence suggests that IFN-β may also act directly on neural stem/progenitor cells (NPCs) in the central nervous system (CNS). NPCs can differentiate into all neural lineage cells, which could contribute to the remyelination and repair of MS lesions. Understanding how IFN-β influences NPC physiology is critical to develop more specific therapies that can better assist this repair process. In this study, we investigated the effects of IFN β-1b (Betaseron®) on human NPCs in vitro (hNPCs). Our data demonstrate a dose-dependent response of hNPCs to IFN β-1b treatment via sustained proliferation and differentiation. Furthermore, we offer insight into the signaling pathways involved in these mechanisms. Overall, this study shows a direct effect of IFN β-1b on hNPCs and highlights the need to further understand how current MS treatments can modulate endogenous NPC populations within the CNS.

1. Introduction

Multiple sclerosis (MS) is an autoimmune disease characterized by multifocal inflammatory lesions in the central nervous system (CNS). Interferon-beta (IFN-β) is a mainstay therapy for MS and has been shown to reduce relapse rate, decrease MRI lesion load, and hinder the rate of brain atrophy (Panitch et al., 2002; Rudick et al., 1999; Schwid et al., 2005). These effects have been attributed to a peripheral immunomodulatory mechanism, including the inhibition of T cell proliferation and a shift from pro-inflammatory to anti-inflammatory cytokines (Rep et al., 1999; Rudick et al., 1996). However, debates over the optimal dose and route of administration remain (Barbero et al., 2004; Barbero et al., 2006; Buchwalder et al., 2000; Coyle and Hartung, 2002; Deisenhammer et al., 1999; Panitch et al., 2002), as the physiologic response to chronic IFN-β treatment is still being characterized. For example, the production of neutralizing and binding antibodies in the periphery may limit the bioavailability of IFN-β (Antonelli and Dianzani, 1999).

Though the blood brain barrier (BBB) may limit IFN-β access to the CNS (Kraus and Oschmann, 2006), IFN-β undoubtedly plays a modulatory role within the CNS as endogenous IFN-β levels rise in the CSF of untreated MS patients (Degré et al., 1976). IFN-β both reduces antigen presentation and decreases the production of pro-inflammatory cytokines by CNS-resident cells (Kawanokuchi et al., 2004; Liuzzi et al., 2004). Alternative routes of IFN-β administration have shown considerable promise in increasing IFN-β levels within the CNS, including intrathecal

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Abbreviations: IFN-β, interferon-beta; MS, multiple sclerosis; CNS, central nervous system; hNPC, human neural stem/progenitor cell; β3-tub, β Tubulin III; BBB, blood brain barrier

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doi:10.1016/j.brainres.2011.07.037

injection (Jacobs et al., 1987), reversible osmotic blood–brain barrier disruption (Greig et al., 1988), stem cell-based delivery (Makar et al., 2008) and intranasal (IN) administration in rats and primates (Ross et al., 2004; Thorne et al., 2008). A better understanding of the benefits of increased IFN-β levels in the CNS may provide novel considerations into the debated dosing and route of administration of IFN-β.

IFN-β may act in the CNS via modulation of CNS-resident neural stem/progenitor cell populations (NPCs). Oligodendrocytes, differentiated from NPCs, are capable of repairing damaged brain lesions via remyelination. It is the insufficiency of this remyelination process that leads to MS disease progression (Mews et al., 1998; Prineas et al., 1993). Therefore, identifying how IFN-β influences NPC physiology is of crucial importance in elucidating the potential benefits of directing therapies that increase IFN-β levels in the CNS. Preliminary studies have suggested that IFN-β promotes NPC differentiation and proliferation but are limited to murine cell models (Hirsch et al., 2009; Lum et al., 2009; Wellen et al., 2009). In this study, we investigate the direct effects of IFN β-1b (Betaseron®) on human NPCs in vitro. We demonstrate that IFN β-1b influences hNPC proliferation and differentiation through a complex signaling cascade. Our data suggest a novel role for IFN β-1b in promoting hNPC-derived CNS regeneration and provide insight into the signaling pathways involved in controlling hNPC fate.

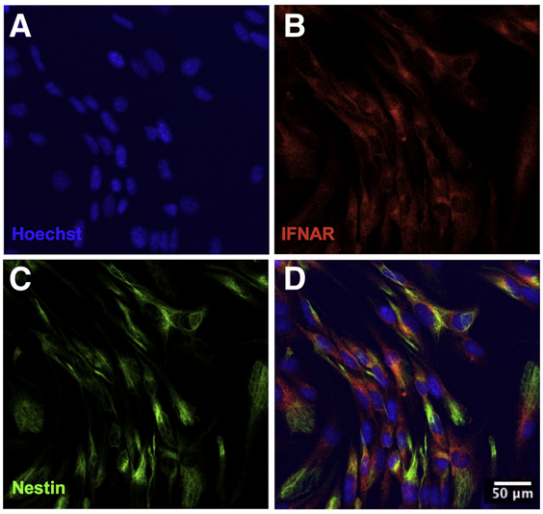

Fig. 1 – hNPCs express the interferon alpha/beta receptor (IFNAR). Human NPCs were stained with the nuclear marker Hoechst (A), IFNAR (B) and the stem cell marker nestin (C) to test for expression of the type I interferon signaling system. Our hNPC line is a pure population of stem cells (C) which express the IFNAR receptor (B). A merge of all stains is shown in (D). The scale bar shown in (D) represents 50 μm and is representative for all 4 panels.

2. Results

2.1. *hNPCs express the IFNAR receptor*

IFN-β is a member of the type-I interferon family and signals through the interferon alpha/beta receptor (IFNAR). We utilized immunocytochemistry to observe if hNPCs express IFNAR. We found that our hNPC population was >98% pure in complete media (i.e. representing a stem-cell population with minimal differentiation), and nearly all cells also stained positive for IFNAR (Fig. 1).

2.2. *IFN β-1b sustains hNPC proliferation*

Activating NPC proliferation represents one means to promote neuro-regeneration by providing a sufficient population of NPCs that can then differentiate into neurons and oligodendrocytes to repair lesions associated with disease. To investigate whether IFN β-1b is capable of influencing proliferation in hNPCs, we grew hNPCs for 1 week in growth factor-deprived minimal media conditions (media devoid of EGF and FGF) with and without treatment of IFN β-1b. IFN β-1b treatment ranged from 1,000–100,000 U/ml. We found that cells grown in the growth factor-deprived minimal media environment have a markedly decreased proliferation rate compared to those grown in media containing 1000–10,000 U/ml IFN β-1b, as demonstrated by an increased ratio of Ki67 positive cells (Fig. 2A and 2B). A similar pro-proliferative effect of IFN β-1b was observed with the BrdU proliferation assay; IFN β-1b treatment at all three concentrations increased the number of BrdU-positive cells as compared to the minimal media control (Fig. 2C).

2.3. *IFN β-1b promotes differentiation of hNPCs*

We further investigated the potential regulatory effects of IFN β-1b on differentiation of hNPCs. Immunocytochemistry analysis showed that lower concentrations of IFN β-1b (1000–10,000 U/ml) had a greater induction of astrocytic marker GFAP and neuronal marker β-III-tub, while higher concentrations of IFN β-1b (100,000 U/ml) demonstrated upregulation of pre-oligodendrocyte marker PDGFRα (Fig. 3A–L). We confirmed differentiation marker expression at the mRNA level by RT-qPCR (Fig. 3M–O), again showing that lower concentrations of IFN β-1b promoted astrocyte cell marker expression, while higher concentrations promoted pre-oligodendroctye cell marker expression. Only a small number of cells stained positive for nestin in minimal media after growth factor deprivation, demonstrating that nearly all cells were committed to differentiation at this 1-week time point (Fig. 3I–K, green).

2.4. *Gene expression array of IFN β-1b treated hNPCs*

SuperArray technology was employed to screen pathway-specific gene expression changes in human NPCs following IFN β-1b treatment. We selected the "Neurotrophins and Receptor Pathway" array in order to further investigate the neuroprotective signaling pathways that may be activated by IFN β-1b. Eight genes had significant fold changes when comparing human NPCs grown in minimal media vs. IFN β-1b treated condition (using a >2-fold change cutoff and P-value<0.05) (Table 1). Of note, STAT1 and STAT2 upregulation confirmed the activation of IFNAR through IFN β-1b binding, as the Jak/STAT