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Stromal cell derived factor-1 acutely promotes neural progenitor cell proliferation in vitro by a mechanism involving the ERK1/2 and PI-3K signal pathways ☆

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Abstract

Stromal cell derived factor-1 (SDF-1), a member of the chemotactic cytokine family, has attracted attention in recent years. It participates in diverse processes such as the regulation of neuronal migration and activation of CD4+ T cells; it is also a co-receptor for human immunodeficiency virus-1 (HIV-1). Here, we show that the proliferation of neural progenitor cells dissociated from rat cortex and cultured in vitro with basic fibroblast growth factor (bFGF) is stimulated by SDF-1. PD98059 and wortmannin, which are, respectively, specific inhibitors of the extracellular regulated kinase1/2 (ERK1/2) and phosphatidylinositol-3 kinase (PI-3K) signal pathways, markedly attenuate this stimulation of proliferation. These findings indicate that SDF-1 acutely promotes the proliferation of NPCs in vitro involving the ERK1/2 and PI-3 kinase pathways, suggesting that it plays a basic role in the development of neural progenitors.

Keywords: Neural progenitor cells (NPCs); SDF-1; Proliferation; ERK1/2; PI-3K

1. Introduction

Neural stem cells (NSC) or neural progenitor cells (NPC) are primordial cells whose potentials for self-renewal and multiple differentiation are the basis of individual neurogenesis. Therefore, an understanding of the proliferation and differentiation mechanisms is of great theoretical value. NPC are also candidates for clinical cell-transplantation treatment of neurodegenerative diseases such as Parkinson's disease, and amplification and purification of these cells in vitro is an important step requiring the participation of neurotrophic factors and cytokines. Nerve growth factor, brain-derived neurotrophin factor, epidermal growth factor, fibroblast growth factor, transforming growth factor-β, etc. have been shown to affect the proliferation and differentiation of NPCs (Benoit et al., 2001; Hagedorn et al., 2000; Temple, 2001; Gritti et al., 1999), and some of these have potential for clinical use.

Chemokines are chemotactic cytokines that activate and direct the immunological reactivity of certain leucocytes (Baggiolini, 1998) and are widely expressed in the human nervous system. One chemokine, SDF-1, has attracted particular recent attention. Its sole receptor, CXC receptor 4 (CXCR4), is highly expressed during embryonic development, but present only at low levels in adults. CXCR4 first appears at scattered sites in the central nervous system (CNS) and pia mater as early as E11 in the rat (McGrath et al., 1999), and its expression during embryonic brain development peaks at E15 in the striatum and cerebral cortex. Expression decreases sharply during the first two postnatal weeks (Lazarini et al., 2002) and is maintained at a low level in normal adult

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ependyma, cortex, hippocampus, cerebellum and brainstem. Its ligand, SDF-1, is also expressed at a low level in adult brain (Ma et al., 1998a; Wong et al., 1996; Banisadr et al., 2000; Lu et al., 2002; Westmoreland et al., 2002). The high expression during embryonic brain development suggests that SDF-1 and its receptor must have roles in early neurogenesis, and it has been shown that SDF-1 is involved in neuronal survival, glial development, cerebellum granular cell migration, etc. (Lazarini et al., 2003). The downstream signaling pathways known to be activated are the ERK1/2 and phospholipase C β pathways (Lazarini et al., 2000; Bajetto et al., 2001).

The temporal expression pattern of SDF-1 and CXCR4 implies roles in early neurogenesis. The proliferation and committed differentiation of NPCs in response to particular stimuli are fundamental processes in neurogenesis. This suggests that SDF-1 may play some part in regulating NPC proliferation or differentiation. We have treated pure NPC cultures in vitro with various agents to test this hypothesis and to identify the relevant signaling pathways.

2. Materials and methods

2.1. Isolation and culture of rat NPCs

To obtain sufficient NPCs, we used E13 Sprague–Dawley rats a little younger than E15 (CXCR4 expression peak) from Peking University Medical School. The NPCs were cultured as previously described (Ma et al., 1998b). Briefly: after mechanical trituration of the cortex and digestion for 15 min at 37 °C with 0.1% trypsin, the suspension was gently triturated, filtered and centrifuged, and the cells were resuspended at a concentration of 10^5 cells per ml in DMEM/F12 (Gibco) with 20 ng/ml bFGF, 1% N2 and 1% B27 (all from Sigma). Half of these amounts of B27 and N2, and the same amount of bFGF, were added every 2 days until 7 days in vitro.

2.2. Immunocytochemical identification of NPCs

NPCs after 7 days' culture were transplanted on poly-D-lysine (PDL)-covered plates, with or without differentiation factors such as 10% fetal bovine serum (Hyclone). We fixed the cells with 4% poly-formaldehyde at the proper time. The first antibodies were mouse polyclonals against nestin (Beijing Yue-Tai), microtubule-associated protein 2 (MAP2, Sigma) and glial fibrillary acid protein (GFAP, Sigma). The second antibody was rhodamine-labeled anti-mouse (Sigma), and DAPI (Sigma) was used as a nucleus-specific dye. Photographs were taken with an OLYMUS XI70.

2.3. MTT cell proliferation test

NPCs starved of bFGF for 24 h were plated evenly on 96-well plates at about 10^5 cells per ml. The plates were treated as follows: no treatment (control), 20 ng/ml bFGF, 50 ng/ml SDF-1, 100 ng/ml SDF-1 and 350 ng/ml SDF-1. After 3 days an MTT assay was performed. Using a Celltiter 96 non-radioactive cell proliferation assay system (Promega), 15 μl dye solution was added to each well and the plates were incubated at 37 °C for 4 h in a humidified CO_2 incubator. Stop solution (100 μl) was added to each well and after 1 h the well contents were mixed and the absorbance of the colored formazan product was measured at 550 nm using a 96 well plate reader (Bio-RAD Bench Mark microplate reader).

2.4. SDF-1 treatment with antagonists

Cells starved of bFGF for 24 h were evenly distributed into six groups, which were treated as follows: no treatment, 20 ng/ml bFGF, 100 ng/ml SDF-1, 100 ng/ml SDF-1 with pre-addition of 50 μM PD98059 for 45 min, 100 ng/ml SDF-1 with pre-addition of 50 nM wortmannin for 30 min, and 100 ng/ml SDF-1 with both PD98059 and wortmannin. Proliferation and other variables were measured after 3 days.

2.5. TUNEL assay

Apoptosis in each group was measured by the TUNEL assay. The cells were attached to PDL-covered plates and fixed with 4% poly-formaldehyde, and then the TUNEL reagents were added following the manufacturer's instructions (DAKO) and before treatment with DAB. Photographs were taken under light microscopy, the dark brown apoptotic cells and the total cells were counted and the apoptosis ratio was determined.

3. Results

3.1. bFGF accelerates NPC proliferation in vitro

As a known mitogen for neural stem cells and many other cell types, 20 ng/ml bFGF markedly increased the proliferation of the E13 cortex NPCs. After primary culture for 5 days, neurospheres were observed. Some of these were transferred on to PDL-covered plates for 1 h and subjected to immunohistochemical examination (Fig. 1). The neurospheres expressed the NPC marker nestin. When differentiation-inducing factors such as serum were added, they differentiated into neurons and glial cells expressing the respective markers MAP2 and GFAP. These results show that bFGF amplifies and purifies the NPCs, which is consistent with previous studies (Temple, 2001; Gritti et al., 1999).

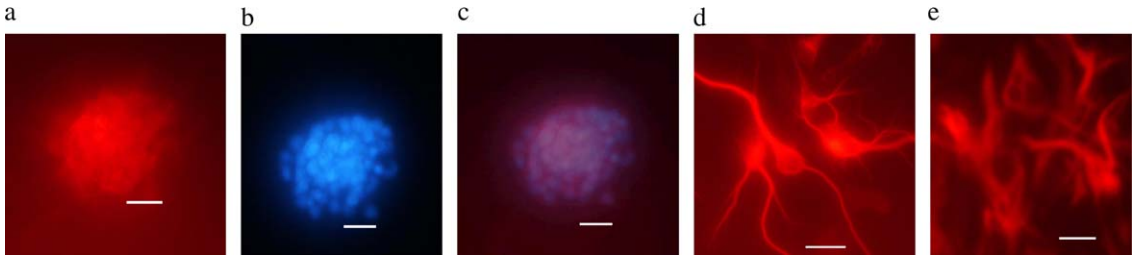

Fig. 1. NPC identification. OlympusXL70, ×40. (a) excitation wavelength 555 nm; red indicates the fluorescence of the rhodamine-labeled second antibody reflecting the distribution of nestin; (b) excitation wavelength 325 nm; blue indicates the nucleus-specific dye DAPI reflecting total cell number; (c) merged with ADOBE PHOTOSHOP; (d) red indicates distribution of MAP2, a neuronal marker; (e) red indicates distribution of GFAP, a glial marker. Scale bar: 30 μm.