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BRAIN RESEARCH

Research Report

Chemokine-like factor 1, a novel cytokine, induces nerve cell migration through the non-extracellular Ca^{2+}-dependent tyrosine kinases pathway

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ABSTRACT

Chemokine-like factor 1 (CKLF1) is a newly cloned chemotactic cytokine. The roles of CKLF1 in the immune system and the respiratory system have been reported, but its function in the nervous system is still remaining unclear. We aimed to investigate the role of CKLF1 in the nerve cell migration and its regulatory mechanisms. By chemotaxis assays and wound-healing assays, CKLF1 stimulated the migration of SH-SY5Y cells dose-dependently. By immunofluorescence staining, CKLF1 induced actin polymerization. By western blotting, proline-rich tyrosine kinase 2 (PYK2) was phosphorylated at Tyr-402 in response to CKLF1 and this phosphorylation was apparently suppressed by phospholipase C-γ inhibitor U73122, but not extracellular Ca^{2+} chelator EGTA. Furthermore, after transfection of dominant-negative mutant PYK2 plasmid, the chemotaxis upon CKLF1 was significantly attenuated in SH-SY5Y cells. Concluding, CKLF1 stimulates the migration of SH-SY5Y cells dose-dependently by activating non-extracellular Ca^{2+}-dependent tyrosine kinases pathway and inducing actin polymerization.

1. Introduction

Chemokines, a family of chemotactic cytokines, play pivotal roles in the control of leukocyte trafficking (Rostene et al., 2007; Tran and Miller, 2003). Initial research studies of chemokines were confined to the immune system for they were intimately involved in the orchestration of inflammatory responses. However, research over the last decade has intrigued neuroscientists that, not specifically concerned with the immune system, some chemokines may also play important roles in the nervous system (Meucci et al., 1998; Mizuno et al., 2003; Zou et al., 1998).

Chemokine-like factor 1 (CKLF1, GenBank accession no. AF096895) is a novel chemokine isolated from PHA-stimulated U937 cells (Han et al., 2001) and a novel functional ligand for the receptor chemokine (C-C motif) receptor 4 (CCR4) (Wang et al., 2006). CKLF1 is a very well established as important regulator of the immune system (Han et al., 2001). However, this appears to be only part of the story. It was reported that CKLF1 mRNA was highly expressed in the brain of fetuses, but

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Abbreviations: CKLF1, chemokine-like factor 1; PYK2, proline-rich tyrosine kinase 2; F-actin, filamentous actin; CCR4, chemokine (C-C motif) receptor 4; PYK2-DN, PYK2-dominant-negative; Rac1, Ras-related C3 botulinum toxin substrate 1; Rac1-DN, Rac1-dominant-negative; PLC-γ, phospholipase C-γ; EGTA, ethylene glycol tetraacetic acid; PKC, protein kinase C; SDF-1α, stromal cell-derived factor-1α; CI, chemotactic index; FBS, fetal bovine serum; BSA, bovine serum albumin

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the expression was at the low level in the brain of adults (Han et al., 2001). So we predict that CKLF1 may be necessary in the brain development.

Nerve cell migration plays an important role in brain development such as the migration of nerve cells from their site of origin in the ventricular zone to their final destination (Marin and Rubenstein, 2003; Metin et al., 2008; Nadarajah et al., 2001). Migration disorders during brain development lead to major neurological diseases such as epilepsy and mental retardation (Chevassus-au-Louis and Represa, 1999; Einstein and Ben-Hur, 2008).

To investigate the potential of CKLF1 to function in neuronal development, we need to access whether CKLF1 regulates neuronal cell migration. To accomplish this goal, we have chosen to work with the cell line SH-SY5Y due to SH-SY5Y human neuroblastoma cell line is an N cell (neuroblasts) line thought to have arisen from the neural crest (Biedler et al., 1978; Puglianiello et al., 2000). SH-SY5Y cells have neuroblast-like characteristics and are often used as a model for studying neuronal development (Pahlman et al., 1990). Moreover, SH-SY5Y cells are amenable to cell transfection unlike primary neuronal cells. Of course, the experiments would require further verification on primary neurons and brain tissues finally. The aim of present research was to investigate the role of CKLF1 in regulating the migration of SH-SY5Y cells and the potential regulatory mechanisms.

2. Results

2.1. *CKLF1 regulates the migration of SH-SY5Y cells in a dose-dependent manner*

Directional migration was assayed using a Boyden chamber and indicated by chemotactic index (CI). To investigate the optimal concentration of CKLF1 for chemotaxis, we firstly examined the dose response of SH-SY5Y cells to CKLF1 in the Boyden chamber cell migration system. Chemotaxis to CKLF1 was induced at 20 nM, 200 nM and 2 μM. SH-SY5Y cells exhibited little basal cell migration, whereas they could be induced to migrate by presenting CKLF1 in the lower chamber. The result showed that CKLF1 stimulated the migration of SH-SY5Y cells in a dose-dependent manner with significant effects at 200 nM ($P<0.01$) and 2 μM ($P<0.001$) (Fig. 1).

Then we measured the relative migration distance of SH-SY5Y cells at the time point of 8 h in response to CKLF1 at different concentrations by wound-healing assays (Fig. 2). The migration of SH-SY5Y cells was enhanced after incubation with increasing doses of recombinant human CKLF1. With CKLF1 200 nM ($P<0.05$) and 2 μM ($P<0.001$) pretreatment, the relative distances of cell migration increased to the values about twice and triple as high as the mock-treated cells, respectively. The result was roughly coincident with that of chemotaxis assay.

2.2. *CKLF1 induces actin polymerization*

The effects of CKLF1 on actin cytoskeleton reorganization in SH-SY5Y cells were monitored by immunofluorescence microscopy (Fig. 3). We presented evidence that CKLF1 enhanced cell spanning filamentous actin (F-actin) fibers and lamellipodia formation around the cell peripheries. Statistical significance of F-actin-stained cells upon CKLF1 stimulation relative to the unstimulated control ($P<0.01$) was determined by one-way analysis of variance (ANOVA).

2.3. *CKLF1 induces tyrosine phosphorylation of PYK2 at Tyr-402*

As shown in Fig. 4, immunoblotting analysis showed that CKLF1 stimulation at 200 nM elevated the phosphorylation of Proline-rich tyrosine kinase 2 (PYK2) at Tyr-402. This result strongly suggests that CKLF1-induced cell migration is potentially related to the activation of PYK2 at Tyr-402 in SH-SY5Y cells.

2.4. *PYK2-dominant-negative mutant or Rac1-dominant-negative mutant attenuates chemotaxis in response to CKLF1*

To gain more insight into the mechanism through which PYK2 and its downstream molecular Ras-related C3 botulinum toxin substrate 1 (Rac1) mediate cell migration stimulated with CKLF1, we examined the effects of overexpression of PYK2-dominant-negative mutant (PYK2-DN, D567N) or Rac1-dominant-negative mutant (Rac1-DN, N17) on CKLF1-induced chemotaxis depicted in Fig. 5A. As shown in Fig. 5B, CKLF1 at 200 nM induced a significant transwell migration of SH-SY5Y cells in comparison with CKLF1-untreated cells ($P<0.001$), whereas cell migration was significantly impaired upon CKLF1 stimulation in D567N ($P<0.001$) or N17 ($P<0.001$) transfected cells compared with pcDNA 3.1 vector transfected control cells.

2.5. *Inhibition of Src or PLC-γ blocks CKLF1-induced tyrosine phosphorylation of PYK2 at Tyr-402*

We finally determined the signaling mechanisms regulating the phosphorylation of PYK2 induced by CKLF1. SH-SY5Y cells were preincubated with various inhibitors, followed by CKLF1 stimulation and western blotting analysis as described. As shown in Fig. 5, CKLF1 induced a strong increase in phosphotyrosine content of PYK2. PYK2-pY402 phosphorylation rose by nearly 9-fold compared with its basal level ($P<0.001$). While in the presence of salicylate (an inhibitor for PYK2) ($P<0.05$), PP2 (a specific inhibitor for Src) ($P<0.05$) and U73122 (a specific inhibitor for PLC-γ) ($P<0.05$), the phosphorylation levels of PYK2-pY402 were significantly attenuated. We also assessed whether extracellular-Ca^{2+} signaling participated in CKLF1-stimulated phosphorylation of PYK2 at Tyr-402. No apparent changes in CKLF1-stimulated phosphorylation at PYK2 Tyr-402 were observed in EGTA-treated cells (PYK2-p402 levels changes by only 2%, Fig. 6).

3. Discussion

Targeted cell migration represents one of the key processes in brain development. The identification of molecules guiding neuronal migration during brain development is crucial for our understanding of brain function. Moreover, unraveling the molecular mechanisms in the developing brain might help to define factors promoting regeneration after damage in the