EXPERIMENTAL CELL RESEARCH 318 (2012) 1889–1900

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/yexcr

Research Article

Morphological phenotypes of olfactory ensheathing cells display different migratory responses upon Slit-2

Ying Wang[a], *Zhi-hui Huang*[b,*]

[a]*School of Laboratory Medicine and Life Science, Wenzhou Medical College, Wenzhou, Zhejiang 325035, China*
[b]*Institute of Hypoxia Medicine, Wenzhou Medical College, Wenzhou, Zhejiang 325035, China*

ARTICLE INFORMATION

Article Chronology:
Received 6 January 2012
Received in revised form
11 May 2012
Accepted 24 May 2012
Available online 5 June 2012

Keywords:
Olfactory ensheathing cells
Migratory properties
Slit-2
Adaptation
Collapse

ABSTRACT

Olfactory ensheathing cells (OECs) are a type of glial cells with morphological plasticity in the olfactory system. Cultured OECs display the process-bearing and flattened shape. Our previous studies have shown that the frontal application of Slit-2 gradient induced the collapse of leading front, and reversed the soma translocation of process-bearing OECs. However, the migratory properties of flattened OECs upon Slit-2 gradient remain elusive. Here, we found that Slit-2 gradient induced the collapse of their plasma membrane, and inhibited migration of flattened OECs. Upon to Slit-2 gradient, the leading front of flattened type 1 OECs firstly showed collapse and retraction, then gradually re-grew a new lamellipodia, finally, showed collapse again (this phenomenon was called as adaptation), while flattened type 2 OECs only showed collapse of plasma membrane. These different migratory responses upon Slit-2 stimulation were possibly due to their different sub-cellular distribution of Robo receptor. Furthermore, F-actin at the peripheral region of leading front was more sensitive to the Slit-2 stimulation than microtubules and the loss of F-actin might be implicated in initiating the collapse of flattened OECs. Finally, the adaptation of flattened type 1 OECs induced by Slit-2 was independent on protein synthesis. Taken together, these results demonstrate that morphological phenotypes of OECs display different migratory properties upon Slit-2 and an unexpected finding that the protein synthesis-independent adaptation in OECs induced by Slit-2.

Introduction

Olfactory ensheathing cells (OECs) are a unique type of glial cells in the olfactory system. These cells are present in both the peripheral nervous system (olfactory epithelium) and central nervous system (olfactory bulb) and share some features and functions with astrocyte and Schwann cells [1,2]. They have been discovered to promote the growth and targeting of olfactory sensory axons during development and the regeneration of injured axons after being transplanted into nerve injury sites [3–6].

OECs display highly variable morphologies *in vivo* and *in vitro* [1,2]. In the olfactory system, OECs appear to have two distinct cytoplasmic electron densities and exhibit elongated and rounded shape [7,8]. The morphology of OECs *in vitro* has been identified in culture from the olfactory epithelium, lamina propria, olfactory nerve, and outer olfactory bulb layer. Cultured OECs display the

Abbreviations: OECs, olfactory ensheathing cells; OE, olfactory epithelium; OB, olfactory bulb; FBS, fetal bovine serum; PLL, poly-L-lysine; LPA, lysophosphatidic acid; L15, Leibovitz's L-15 medium; Aniso, anisomycin; CHX, cycloheximide; CM, control medium; RoboN, medium containing ectodomain of Robo-1.

*Corresponding author.
E-mail address: hzhzju021@163.com (Z.-h. Huang).

0014-4827/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yexcr.2012.05.024

long fusiform bipolar or multi-polar morphology (process-bearing shape) and the flat sheet-like morphology (flattened shape) [2,9–16]. The morphology of OECs is affected by some extracellular factors such as cultured media, endothelin-1 [10] and extracellular matrix [17], and intracellular factors such as cAMP [9,10,18]. Interestingly, several studies have shown that reversible changes have been found between these two shapes based on time-lapse imaging [9,19] or addition of cAMP or endothelin-1 [10]. These studies support the notion that OECs are a single cell type with morphological plasticity [1]. Our recent studies have shown that RhoA-ROCK-Myosin pathway regulates the morphological plasticity of cultured OECs [20]. However, the cellular properties of two morphological phenotypes still remain elusive.

During the development of olfactory system, OECs migrate out of the olfactory epithelium (OE) together with growing olfactory sensory axons from the lamina propria to the presumptive olfactory bulb (OB) [8,21–23]. In the migratory pathway from OE to OB, OECs have to interact with extracellular matrix and factors produced by surrounding tissues. Stimulation of OEC motility enhances olfactory axon growth [24,25]. In OEC transplantations, OECs also have to face a more complex environment during their migration, as they interact with a great variety of cell types such as reactive astrocytes and with the factors produced through injury [26–28]. Slits and Robos are a pair of conserved repulsive ligands and receptors for axon pathfinding and cell migration [29–33]. In the olfactory system, Slits and Robos are expressed in a specific spatio-temporal pattern, and play important roles in the guidance of olfactory sensory axons [34,35]. Moreover, Slits are highly expressed in injury nervous system [36–40]. Our recent studies have shown that Slit-2 repels the migration of process-bearing OECs through calcium-dependent cofilin activation and RhoA inhibition [41]. However, the migratory properties of flattened OECs upon Slit-2 remain elusive. In the present study, we found that Slit-2 gradient induced the collapse, adaptation and inhibited the migration of flattened OECs.

Materials and methods

Primary culture and purification of olfactory ensheathing cells

Primary OEC cultures were prepared from olfactory bulb of adult male Sprague-Dawley rats and purified by differential cell adhesiveness as described previously [9,42]. Briefly, the meninges were carefully removed from the olfactory bulb under the dissecting microscope and the olfactory nerve layer was peeled away from the glomerular and deeper layers of the olfactory bulb, then dissociated with 0.125% trypsin (Sigma, St Louis, MO) and incubated at 37 °C for 15 min. Trypsinization was stopped by DMEM/F12 (1:1, vol/vol, Gibco, Grand Island, NY) containing 10% heat-inactivated fetal bovine serum (FBS, Hyclone, Logan, UT). The tissue was centrifuged for 10 min at 500 g, and the pellet was triturated using a flame-polished Pasteur pipette and plated on uncoated 25 cm^2 culture flask (Corning, LY) two times, each for 36 h at 37 °C in 5% CO_2. The non-adhesive cell suspension was collected and then seeded onto 12-well plates (Corning) pre-coated with poly-L-lysine (PLL, 0.1 mg/ml, Sigma), and incubated with DMEM/F-12 containing 10% FBS, 2 μM forskolin (Sigma) and 10 ng/ml bFGF (Sigma) as mitogen. The media were changed every 3 days. The overall purity of OECs was around 95%. The definition of OEC phenotypes is described previously [9,10]. Briefly, process-bearing OECs had very little cytoplasm and two or more fine processes that were longer than the width of the cell body and flattened OECs had a large area of cytoplasm surrounding the nucleus and either fewer than two processes or processes that were shorter than the width of the cell body. The latter could be further divided into two subtypes according to the location of the nucleus. Flattened type 1 OECs were defined as those exhibiting a fan-like shape, with a nucleus lying at the edge of the cytoplasm and a large lamellipodium extending from the opposite side to the nucleus. Flattened type 2 OECs were defined as those having a round shape and a nucleus lying at the center of the cytoplasm.

Slit-2 purification

Slit-2 was purified as described previously from conditioned medium collected from a cultured HEK293 cell line stably expressing full-length human Slit-2-myc [41,43]. The protocol of Slit-2-myc purification was modified from the protocol provided by Covance. Briefly, we firstly collected and condensed more than 100 kD proteins from conditioned medium of Slit-2-myc by an Amicon Ultra-15 Centrifugal Filter (Millipore). Secondly, we pulled down and purified the Slit-2-myc factor by using the 9E10 monoclonal anti-myc antibody affinity matrix (Covance) from conditioned medium. Finally, we detected the purified Slit-2-myc by western blot using anti-myc antibody (1:1000, Cell Signaling Technology) (Fig. S1A). Purified Slit-2 could induce the collapse of growth cone of cultured cerebellum granule neuron (Fig. S1B–C).

Single-cell migration assays

Single OEC migration assay and quantitative analysis were carried out as described previously [9,41]. Briefly, the purified OECs were re-plated onto square coverslips (8 mm) coated with laminin (10 μg/ml) at a low density of about 1000 cells per coverslip. 24 h after plating, the coverslip was put into a chamber containing 1 ml serum-free Leibovitz's L-15 medium (L15, Gibco). The chamber was then covered with a thin layer of methyl-siloxane fluid to prevent medium evaporation. The experiments were carried out at heated stage (37 °C) of a phase contrast microscope (CK40, Olympus optial, Tokyo, Japan). Selected cell was with a typical OEC morphology and without attached with any other cells. Micropipettes used for pulsatile ejection were pulled with a two-stage puller designed for making patch-clamp electrodes. A micropipette with a tip opening of about 1 μm was placed at 15 μm perpendicular and 100 μm away from the center of the tested cell. A standard pressure pulse of 3 psi (1 psi=6.89 kPa) in amplitude and 20 ms in duration, was generated by a pulse generator and applied to the micropipette at a frequency of 2 Hz. Under this standard condition, the concentration of factor at 100 μm from micropipette tip is about 10^{-3} fold lower than that in the micropipette [44]. Images of OECs were recorded, in a time-lapse mode (one picture/5 min interval), with a CCD camera (JVC TK-1381; Victor Company, Yokohama, Japan) attached to the microscope and stored in a computer for further analysis using Scion image software (Frederock, MD) [9]. Briefly, we measured the distance of cell migration during a control period and after treatment, and calculated the respective migration rates (distance/time), then calculated the migration ratio (migration rate after treatment/