Available online at www.sciencedirect.com
SciVerse ScienceDirect
www.elsevier.com/locate/brainres

Research Report

Olfactory ensheathing cells of hamsters, rabbits, monkeys, and mice express α-smooth muscle actin ☆

Khalil S. Rawji[a], Shannon X. Zhang[b], Ying-Yu Tsai[b], Laura J. Smithson[a], Michael D. Kawaja[a,b,*]

[a]Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada K7L 3N6
[b]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada K7L 3N6

ARTICLE INFO

Article history:
Accepted 2 May 2013
Available online 9 May 2013

Keywords:
S100β
Glial fibrillary acidic protein
α-smooth muscle actin
Mammalian glia
Olfactory mucosa
Olfactory bulb

ABSTRACT

Olfactory ensheathing cells (OECs) are the chief glial population of the mammalian olfactory nervous system, residing in the olfactory mucosa and at the surface of the olfactory bulb. We investigated the neurochemical features of OECs in a variety of mammalian species (including adult hamsters, rabbits, monkeys, and mice, as well as fetal pigs) using three biomarkers: α-smooth muscle actin (αSMA), S100β, and glial fibrillary acidic protein (GFAP). Mucosal and bulbar OECs from all five mammalian species express S100β. Both mucosal and bulbar OECs of monkeys express αSMA, yet only bulbar OECs of hamsters and only mucosal OECs of rabbits express αSMA as well. Mucosal OECs, but not bulbar OECs, also express GFAP in hamsters and monkeys; mice, by comparison, have only a sparse population of OECs expressing GFAP. Though αSMA immunostaining is not detected in OECs of adult mice, GFAP-expressing mucosal OECs isolated from adult mice do coexpress αSMA *in vitro*. Moreover, mucosal OECs from adult mutant mice lacking αSMA expression display perturbed cellular morphology (*i.e.*, fewer cytoplasmic processes extending among the hundreds of olfactory axons in the olfactory nerve fascicles and nuclei having degenerative features). In sum, these findings highlight the efficacy of αSMA and S100β as biomarkers of OECs from a variety of mammalian species. These observations provide definitive evidence that mammalian OECs express the structural protein αSMA (at various levels of detection), which appears to play a pivotal role in their ensheathment of olfactory axons.

1. Introduction

The mammalian olfactory nervous system is known for its ability to continually generate new primary olfactory neurons throughout adulthood (Graziadei and Graziadei, 1979). This neurogenesis occurs in the neuroepithelium, where the cell bodies of primary olfactory neurons and their basal stem cells are located. The olfactory mucosa is composed of this

☆Support: This work was supported by a grant from the Botterell Foundation at Queen's University. Khalil S. Rawji is supported by an Ontario Graduate Scholarship and Laura J. Smithson is supported by a Banting and Best Scholarship from the Canadian Institutes of Health Research.

*Correspondence to: Department of Biomedical and Molecular Sciences, Room 947 Botterell Hall, Queen's University, Kingston, Ontario, Canada K7L 3N6. Fax: +1 613 533 2022.
E-mail address: kawajam@queensu.ca (M.D. Kawaja).

0006-8993/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2013.05.003

superficial avascular neuroepithelium and a deeper (highly vascularized) layer referred to as the lamina propria. The primary olfactory neurons extend small unmyelinated axons (0.1–0.4 μm in diameter) through the lamina propria toward the olfactory bulbs. Specialized glial cells known as olfactory ensheathing cells (OECs) protect the olfactory axons in the lamina propria by creating tunnel-like structures that surround dozens to hundreds of fibers simultaneously (Doucette, 1990). These OEC fascicles ensheathing the olfactory axons continue through the cribriform plate and terminate at the surface of the olfactory bulbs, where these axons form the olfactory nerve fiber layer. Rather than clearly-defined fascicles, OECs in the olfactory nerve fiber layer cluster together to surround crisscrossing bundles of olfactory axons; these axons extend into the olfactory bulbs where they synapse with the dendritic processes of the second-order olfactory mitral, periglomerular, and tufted neurons in the glomerular layer (Valverde et al., 1992; Franssen et al., 2007).

Over the last 15 years, studies have shown that cultures containing OECs isolated from rat and mouse olfactory tissues can be successfully used for implantation into the injured spinal cord of adult rats. These studies have reported that such cellular implants can reduce spinal cavitation, promote axonal regeneration, and result in a modest degree of functional motoric recovery (Ramon-Cueto and Valverde, 1995; Franklin and Barnett, 2000; Bunge, 2001; Raisman, 2001; Boyd et al., 2003; Ruitenberg et al., 2006; Richter and Roskams, 2008). One outstanding issue of this cellular strategy has been the unequivocal identification of OECs in these cultures of olfactory tissue, which have been used for spinal implantation (Boyd et al., 2004, 2006). One approach toward resolving this problem has been through the identification of biomarkers that can successfully distinguish OECs from other phenotypically similar glial cells, such as Schwann cells. Using a proteomic strategy to examine the phenotypes of rat OECs, our group has shown that both mucosal and bulbar OECs from rats express certain structural proteins, including calponin and α-smooth muscle actin (αSMA) *in vivo* and *in vitro*; two proteins which are not expressed by Schwann cells (Boyd et al., 2006; Rizek and Kawaja, 2006; Jahed et al., 2007; Kawaja et al., 2009). That OECs are immunopositive for αSMA has been validated by revealing similar detection in OECs of guinea pigs and cats (Smithson and Kawaja, 2009). While the expression of glial biomarkers such as S100β and glial fibrillary acidic protein (GFAP) by mammalian OECs *in situ* has been widely accepted, it seems quite unexpected that OECs from rats, guinea pigs, and cats would also express a structural protein like αSMA. We have speculated that αSMA confers a degree of structural plasticity to OECs as they ensheath variable numbers of olfactory axons from the most distal olfactory mucosa to the olfactory nerve fiber layer around the olfactory bulb. Unlike non-myelinating Schwann cells that associate with a dozen or so small-diameter sensory or autonomic axons, OECs can be seen ensheathing a great range of olfactory axon numbers—from a few dozen to many hundreds. Moreover, while the numbers of unmyelinated peripheral axons associated with each Schwann cell remains relatively static throughout life, the numbers of unmyelinated olfactory axons associated with each OEC is constantly changing due to the degeneration and generation of new olfactory neurons.

In this study, we sought to provide further evidence that mucosal and bulbar OECs express αSMA in a variety of mammalian species and that αSMA, along with S100β and GFAP, confers a unifying phenotype for these glial cells *in vivo*. Here we examined the neurochemical features of OECs in adult hamsters, rabbits, monkeys, and mice, as well as in fetal pigs. By comparing biomarkers for OECs across multiple mammalian species, we have shown here and in a previous study (Smithson and Kawaja, 2009) that the OEC phenotype can, in fact, be defined as glial cells expressing S100β with variable levels of αSMA. Though levels of αSMA elude immunodetection in adult mice *in vivo*, we offer two observations that support our postulate that murine OECs express αSMA: (1) GFAP-expressing mucosal OECs, isolated from wild type mice, display positive immunostaining for filamentous αSMA *in vitro*; and (2) mucosal and bulbar OECs, examined in mice lacking functional αSMA expression, have features indicative of structural defects in the absence of this filamentous protein. In sum, these new data support the idea that mammalian OECs express αSMA, which appears to play a critical role in the structural integrity of these glial cells and in their important protective association with olfactory axons.

2. Results

The anatomical organization of the adult hamster, rabbit, non-human primate, and mouse (as well as fetal pig) olfactory nervous systems is quite similar to that of the adult rat, guinea pig, and cat. The olfactory mucosa has olfactory nerve fascicles within the lamina propria layer that increase in size as they near the olfactory bulbs. These olfactory nerve fascicles are composed of OECs wrapping around variable numbers of olfactory axons. Though the exact morphological arrangement between OECs and olfactory axons varies among mammalian species (see Kawaja et al., 2009), it is clear that the primary function of OECs is to ensheath dozens (if not hundreds) of olfactory axons. Each fascicle is bound by a layer of perineurial fibroblasts, and in certain species, the larger olfactory nerve fascicles contain a single arteriole. At the olfactory nerve fiber layer, the fascicular arrangement of OECs and olfactory axons is replaced by clusters of OECs that surround bundles of criss-crossing olfactory fibers.

In the olfactory mucosa of adult hamsters, the cytoplasmic processes of OECs in olfactory nerve fascicles displayed positive immunostaining for both GFAP and S100β (Fig. 1). Though hamster bulbar OECs also had positive immunostaining for S100β, there was a pronounced lack of GFAP immunostaining among these cells. Positive immunostaining for GFAP (and S100β) was, however, evident in the astrocytes of the olfactory bulb. Immunostaining for αSMA of the hamster olfactory mucosa revealed vascular smooth muscle cells (VSMCs) of arterioles (some of which were present within the larger olfactory nerve fascicles) and perineurial fibroblasts; this positive immunostaining was seen without pretreatment with proteinase K. Perineurial fibroblasts (*i.e.*, flattened cells surrounding the nerve fascicles) have been described in the olfactory mucosa of adult rats, cats, and guinea pigs (Jahed et al., 2007; Kawaja et al., 2009; Smithson