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BRAIN RESEARCH

Research Report

Similar behaviour and primate-like properties of adult canine Schwann cells and olfactory ensheathing cells in long-term culture

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ABSTRACT

Adult canine Schwann cells and olfactory ensheathing cells (OECs) have been shown to promote neural regeneration *in vivo*. Since the majority of studies have been performed in rodents, it is not yet clear in how far OECs from large animals and humans share the reported properties. Moreover, due to the lack of comparative studies, it remains to be established whether Schwann cells and OECs display cell type-specific characteristics. In the present study, adult canine Schwann cells and OECs were comparatively analyzed regarding long-term growth, morphology, growth factor responsiveness, and antigenic expression. Adult canine Schwann cells and OECs displayed the same typical spindle-shaped morphology and expressed the cell type-specific marker $p75^{NTR}$. Moreover, the proliferation of both cell types was promoted by the same mitogens, including fibroblast growth factor-2 (FGF-2) and heregulin-1β (HRG-1β). Several observations indicate that canine OECs differ from the well characterized rodent OECs and display properties reminiscent on primate cells. Both cell types (i) proliferated through multiple passages in the absence of growth factors and did not enter a senescent state until 3 months in culture, (ii) were not responsive to the cAMP-elevating agent forskolin, and (iii) stably expressed $p75^{NTR}$ in long-term culture. Taken together, this is the first report demonstrating that adult canine Schwann cells and OECs in long-term culture share the same *in vitro* characteristics and display primate-like properties. This underscores the relevance of the dog as a translational species between rodents and humans.

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Abbreviations: BrdU, 5-Bromo-2′-deoxy-uridine; CNTF, Ciliary neurotrophic factor; dbcAMP, dibutyryl cyclic adenosine monophosphate; DME, Dulbecco's modified Eagle; EGF, Epidermal growth factor; ELISA, Enzyme-linked immunoadsorbent assay; FCS, Fetal calf serum; FGF-2, Recombinant human fibroblast growth factor-2; GFAP, Glial fibrillary acidic protein; HCl, Hydrogen chloride; HRG-1β, Heregulin-1β; MACS, Magnet-activated cell separation; OECs, Olfactory ensheathing cells; $p75^{NTR}$, p75 neurotrophin receptor; PBS, Phosphate buffer saline; PBST, PBS–Triton-X100; PLL, Poly-L-lysine

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1. Introduction

Schwann cells and olfactory ensheathing cells (OECs) are closely-related glial cells that have been shown to promote neural repair following transplantation into the lesioned nervous system (Honmou et al., 1996; Ramón-Cueto et al., 1998; Barnett et al., 2000; Kohama et al., 2001; Wewetzer et al., 2002; Akiyama et al., 2004). Based on these properties, both cell types are considered promising candidates for cell transplantation-based therapies of human CNS injury and disease (Santos-Benito and Ramón-Cueto, 2003; Barnett and Riddell, 2007; Lavdas et al., 2008). However, the vast majority of the studies have been performed in the rodent model and it is not clear in how far the obtained data can be extrapolated to humans (Wewetzer and Brandes, 2006). Although the adult dog has been recently introduced as a translational model for the study of spinal cord repair and *in vivo* studies reported beneficial effects of adult canine OECs (Smith et al., 2001; Jeffery et al., 2005, 2006; Ito et al., 2006; Krudewig et al., 2006), little is known about the *in vitro* properties of OECs and their characterization has remained fragmentary. Even less is known about the biology of canine Schwann cells, whose purification has been described in a single report (Pauls et al., 2004). Thus, the question of which cell type is most suitable for CNS repair, which is discussed controversially in the rodent model, is also open for the canine system. The assumption of species-specific properties of Schwann cells and OECs is underscored by recent studies. In contrast to rat OECs, adult human and canine OECs do not respond to forskolin with proliferation and transition of their morphology (Barnett et al., 2000; Krudewig et al., 2006). Moreover, primate OECs but not rodent OECs display a prolonged life span *in vitro* and a stable expression of the neurotrophin receptor p75 (Rubio et al., 2008).

In the present study, we focused on the comparative analysis of adult canine Schwann cells and OECs in order to lay the groundwork for the transplantation of both cell types

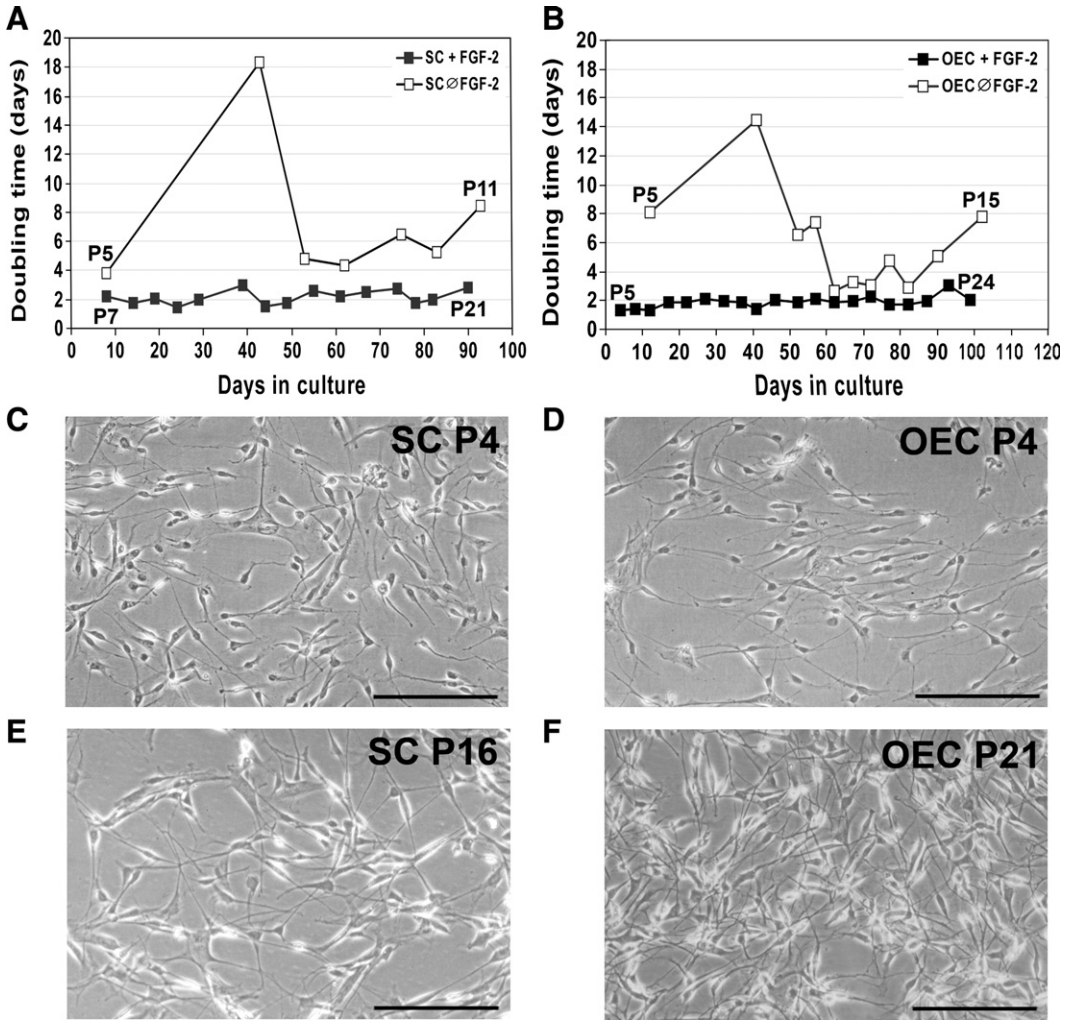

Fig. 1 – Long-term *in vitro* growth and morphology of adult canine Schwann cells (SC, A, C, E) and olfactory ensheathing cells (OEC, B, D, F) in the presence (+ FGF-2) and absence of FGF-2 (ø FGF-2). During the 3-month-culturing period, single clones of FGF-2-treated Schwann cells and OECs displayed shorter population doubling times than untreated cells throughout multiple passages (P) (A, B). Morphology of Schwann cells (P4: C and P16: E) and OECs (P4: D and 21: F) in the absence of FGF-2. Both glial cell types displayed the typical bi- to tripolar shape and did not change upon FGF-2 treatment up to P25. Scale bar ~ 200 μm.