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Review

Species-specific control of cellular proliferation and the impact of large animal models for the use of olfactory ensheathing cells and Schwann cells in spinal cord repair

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ABSTRACT

Autologous transplantation of olfactory ensheathing cells (OECs) and Schwann cells (SCs) is considered a promising option to promote axonal regrowth and remyelination after spinal cord injury in humans. However, if the experimental data from the rodent model can be directly extrapolated to humans, as widely believed, remains to be established. While limitations of the rodent system have recently been discussed with regard to the distinct organization of the motor systems, the question whether OECs and SCs may display species-specific properties has not been fully addressed. Prompted by recent studies on canine and porcine glia, we performed a detailed analysis of the *in vitro* and *in vivo* properties of OECs and SCs and show that rodent but not human, monkey, porcine, and canine glia require mitogens for *in vitro* expansion, display a complex response to elevated intracellular cAMP, and undergo spontaneous immortalization upon prolonged mitogen stimulation. These data indicate fundamental inter-species differences of the control of cellular proliferation. Whether OECs and SCs from large animals and humans share growth-promoting *in vivo* properties with their rodent counterpart is not yet clear. Autologous implantation studies in humans did not reveal adverse effects of cell transplantation so far. However, *in vivo* studies of large animal or human glia and rodent recipients mainly focused on the remyelinating potential of the transplanted cells. Thus, further experimental *in vivo* studies in large animals are essential to fully define the axonal growth-promoting potential of OECs and SCs. Based on the homology of the *in vitro* growth control between porcine, canine and human glia, it is concluded that these species may serve as valuable translational models for scaling up human procedures. This article is part of a Special Issue entitled: Understanding olfactory ensheathing glia and their prospect for nervous system repair.

Contents

Introduction

Many different *in vivo* studies have provided evidence that implantation of olfactory ensheathing cells (OECs) and Schwann

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cells (SCs) into the lesioned central and peripheral nervous system promotes both axonal regeneration and remyelination (Lankford et al., 2002; Wewetzer et al., 2002; Santos-Benito and Ramón-Cueto 2003; Lavdas et al., 2008). Thus, transplantation of growth-promoting glia, such as OECs and SCs is considered a promising option for the treatment of nervous system injury and disease (Kocsis, 1999; Franssen et al., 2007, Radtke et al., 2010b). However, due to the limited availability of human tissue, the vast majority of studies have been carried out in rodents and very little is known about human OECs and SCs (Radtke and Wewetzer, 2009). Currently, we face a situation where a wealth of experimental data from rodents faces only few clinical phase I studies in humans (Féron et al., 2005; Lima et al., 2006, 2010; Mackay-Sim et al., 2008). So far only a few investigations used glia from large animals to study the regenerative capacity of OECs and SCs within the same experimental paradigm (for review, see Wewetzer et al., 2002; Wewetzer and Brandes, 2006). This is due to the fact that research on the regenerative potential of OECs and its translation into clinical practice in the past has been dominated by two main assumptions. First, it is generally believed that OECs, which are associated *in situ* with olfactory neurons entering the olfactory bulb throughout life-time (Graziadei and Monti Graziadei, 1985, Raisman, 1985) display unique regenerative properties that are superior to the ones of SCs from the peripheral nerve (Ramón-Cueto and Valverde, 1995; Franklin and Barnett, 1997; Lankford et al., 2008; Kocsis et al., 2009). Second, it was hypothesized that human OECs and SCs behave similarly if not identical as their rodent counterpart. As a consequence of the former assumption, *in vivo* effects of OECs were mainly characterized in relation to vehicle solution controls rather than in direct comparison with the closely related SCs (for review, see Wewetzer et al., 2002; Wewetzer and Brandes, 2006).

The fact that putative species-specific effects of OECs and SCs have not been discussed so far is also a result of the second hypothesis. The thorough review of the current literature clearly reveals (see below, Table 1) that primate or human OECs have been regularly maintained so far *in vitro* according to protocols originally established for rodent cells. Instead, it would be important to clarify and investigate independently the importance and relevance of certain factors, such as an elevated intracellular cAMP level, for the *in vitro* growth of primate and human glia. The idea that the rodent data can be extrapolated to the human system also becomes apparent when analyzing the *in vivo* approaches. Considering the experimental data currently available it seems that the gap between the species, rodents and humans, is at least as relevant as the literal gap in spinal cord injury.

To translate the rodent data into clinical practice, it is essential to do a comparative analysis between the species and to answer two major questions. First, are there any differences in the organization of the motor systems between rodents and humans relevant to the clinical application of growth-promoting glia? And second, do human OECs and SCs display species-specific properties different from rodents? If the answer is yes, the next step should be the identification of species with closest homology to the human system. Whereas the former issue has been recently covered by Courtine et al. (2007), who recommended the use of nonhuman primates as translational models, a critical and comparative analysis of OECs and SCs has not been done so far. On a methodological level, it is important to analyze the protocols designed for cell isolation since distinct protocols may lead to the cultivation of different cell types resulting in varying results, including cell purity (Kawaja et al., 2009). Another level refers to the functional capacity of the cells and the question, in how far rodent glia

Table 1
Comparative analysis of *in vitro* properties of olfactory ensheathing cells (OECs) and Schwann cells (SCs) from different species. Nd, —— (not done) +/− = controversial results have been provided; * = studies that tested OECs and Schwann cells in parallel, ** = that long-term studies were performed, but no reports on immortalization events were provided.

		Rat	Pig	Dog	Monkey	Human	References
Schwann cell mitogen-induced proliferation	OEC	+	+	+	+	+	Rat: Chuah and Teague (1999); Wewetzer et al. (2001); Yan et al. (2001); pig: Radtke et al. (2010b); dog: Smith et al. (2002); Krudewig et al. (2006); Bock et al. (2007); Techangamsuwan et al. (2008); (2009); monkey: Rubio et al. (2008)*; human: Barnett et al. (2000)
	SC	+	Nd	+	+	+	Rat: Lemke and Brockes (1984); Porter et al. (1986); Davis and Stroobant (1990); Schubert (1992); pig: ––––; dog: Pauls et al. (2004); Techangamsuwan et al. (2008)*, (2009)*; Schmitte et al. (2009); monkey: Avellana-Adalid et al. (1998); human: Rutkowski et al. (1995)*; Monje et al. (2006)
Autonomous growth *in vitro*	OEC	−	+	+	+	+	Rat: Wewetzer et al. (2001); Yan et al. (2001); pig: Radtke et al. (2004); Radtke et al. (2010b); dog: Techangamsuwan et al. (2008)*; primate: Rubio et al. (2008)*; human: Barnett et al. (2000)
	SC	−	Nd	+	+	Nd	Rat: Wood and Bunge (1975); Porter et al. (1986); Rubio et al. (2008)*; pig: ––––; dog: Pauls et al. (2004); Techangamsuwan et al. (2008)*; monkey: Avellana-Adalid et al. (1998); human: ––––
Response to elevated intracellular cAMP	OEC	+	Nd	−	−	−	Rat: Wewetzer et al. (2001); Yan et al. (2001); pig: ––––; dog: Krudewig et al. (2006); Techangamsuwan et al. (2008)*; monkey: Rubio et al. (2008); human: Barnett et al. (2000)
	SC	+	Nd	−	−	+/−	Rat: Raff et al. (1978); Chen et al. (1991);Sobue et al. (1986); Davis and Stroobant (1990); Jessen et al. (1991); Stewart et al. (1991); pig: ––––; dog: Techangamsuwan et al. (2008); monkey: Avellana-Adalid et al. (1998); human: Levi et al. (1995); Rutkowski et al. (1995); Fregien et al. (2004); Monje et al. (2006)
Long-term culture: I. Spontaneous immortalization	OEC	+	Nd	−	−	Nd	Rat: Sonigra et al. (1996); pig: ––––; dog: Krudewig et al. (2006); Techangamsuwan et al. (2008)*; (2009); monkey: Rubio et al. (2008)* human: ––––
	SC	+	Nd	−	(−)**	(−)**	Rat: Eccleston et al. (1991); Goda et al. (1991); Bolin et al. (1992); Watabe et al. (1995); Saravanan et al. (2007); Shen et al. (2002); Ohsawa et al. (2005); pig: –––; dog: Techangamsuwan et al. (2008)*; (2009); monkey: Avellana-Adalid et al. (1998); human: Moretto et al. (1984); Rutkowski et al. (1995); Van den Berg et al. (1995)
Long-term culture: II. proliferation	OEC	−	−	+	+	Nd	Rat: Rubio et al. (2008)*; pig: Radtke et al. (2010b) dog: Krudewig et al. (2006); Techangamsuwan et al. (2008)*; (2009)*; monkey: Rubio et al. (2008)* human: ––––;
	SC	−	Nd	+	+	+/−	Rat: Brockes et al. (1980); Porter et al. (1986); Davis and Stroobant (1990) pig: ––– dog: Techangamsuwan et al. (2008)*; (2009)* monkey: –––– human: Moretto et al. (1984); Rutkowski et al. (1995); Van den Berg et al. (1995)
Long-term culture: III. $p75^{NTR}$ expression	OEC	−	−	+	+	Nd	Rat: Rubio et al. (2008)*; pig: Radtke et al. (2010b) dog: Krudewig et al. (2006); Techangamsuwan et al. (2008)* (2009) monkey: Rubio et al. (2008)* human: ––––
	SC	−	Nd	+	Nd	Nd	Rat: ––– pig: ––– dog: Techangamsuwan et al. (2008)*, (2009)*, monkey:–––––; human: ––––

Table 2
Transplantation of large animal or human OECs into either rodent or large animals and humans. Shaded in light grey and grey are the papers that applied OECs to the lesioned spinal cord of large animals and humans, respectively. (Olfactory bulb OB, olfactory mucosa OM). * = studies that tested the effects of OECs and SCs in parallel.

Lesion model	Host	Graft	Transplant	Morphological analysis	Functional analysis	References
Demyelination	Rodent	Human (Rat)	Disscociated OECs	Increased remyelination	Not performed	Kato et al. (2000)
Demyelination	Rodent	Human	Dissociated OECs, purified	Increased remyelination (Po)	Not performed	Barnett et al. (2000)
Demyelination	Rat	Human	Dissociated OECs purified (SCs)	Increased remyelination	Increased conduction velocity	Imaizumi et al. (2000)*
Demyelination	Rat	Canine	Dissocated OEC, purified	Increased remyelination (Po)	Not performed	Smith et al. (2002)
Intact and injured spinal cord	Rat	Human (Rat)	Dissociated OECs, purified	Survival and migration	Not performed	Deng et al. (2006)
Demyelination	Rat	Porcine	Dissociated OECs purified	Increased remyelination	Not performed	Radtke et al. (2010)
Spinal cord transection	Rat	Monkey	Dissociated OECs, Purified	No long tract CST regeneration	Behavorial recovery Open field	Guest et al. (2008)
Spinal cord injury	Rat	Human	Dissociated OEC	Reduced lesion volume and cavity	Increased hindlimb function (BBB)	Gorrie et al. (2010)
Cerebral ischemia	Rat/Mouse	Human	Dissociated OECs	Increased growth factor expression	Behavorial improvement	Shyu et al. (2008)
SCI phase I trial	Canine	Canine	Dissociated OECs	Not performed	Increased BBB No adverse effects	Jeffery et al. (2005)
Demyelination	Monkey	Porcine	Dissociated OECs, Purified	Increased remyelination	Not performed	Radtke et al. (2004)
SCI phase I trial	Human	Human	Dissoc. OECs	Not performed	No adverse effects	Féron et al. (2005)
SCI phase I trial	Human	Human	OM Explants	Not performed	Increased ASIA Few adverse effects	Lima et al. (2006)
SCI Phase I/IIa trial	Human	Human	Dissociated OECs	Not performed	Functional improvement in 1/6 No adverse effects	Mackay-Sim et al. (2008)
SCI phase I trial	Human	Human	OM Explant	Not performed	Increased EMG, SSEP Few adverse effects	Lima et al. (2010)

reflects the human system and whether large animals are closer related to humans or rodents (Kastner and Gauthier, 2008). The properties of large animal and human OECs and SCs can be determined either by *in vitro* analysis or by implantation of the cells into the rodent or large animal/human spinal cord.

In the present review, we performed a detailed comparative *in vitro* and *in vivo* analysis of published properties of OECs from different species, such as monkeys, dogs, and pigs. Though adult dogs and pigs were initially introduced as models for *in vivo* studies (Imaizumi et al., 2000; Smith et al., 2002, Radtke et al. 2004; Jeffery et al., 2005, 2006), a detailed *in vitro* characterization of the cells has not been done until recently in the dog (Krudewig et al., 2006; Bock et al., 2007; Techangamsuwan et al., 2008, 2009; Haastert et al., 2009; Schmitte et al., 2009) or has just begun as in the pig (Radtke et al., 2010a). The same is true for human cells that have been implanted in the rat or into humans but whose *in vitro* characterization has remained fragmentary. We include SCs in our analysis, since many of the aspects of glial cell properties were originally studied in SCs and the number of papers that focused on the direct comparison of OECs and SCs are still scarce (Angelov et al., 2005; Lankford et al., 2008; Franssen et al., 2009). The present review is divided into two main parts that refer to the *in vitro* and *in vivo* properties of glial cells. Since *in vitro* approaches generally have the inherent advantage of a reduced complexity that facilitates a comparative analysis, the main emphasis is on the *in vitro* behaviour of OECs and SCs from different species, including rats and mice. Special attention was given to the responsiveness to growth factors, *in vitro* proliferation and differentiation together with the susceptibility of the cells to immortalization following sustained mitogen stimulation (Table 1). These criteria were defined, at least in part, on the basis of availability of experimental evidence. Due to space limitations and the fact that *in vivo* properties of rodent OECs and SCs have already been reviewed previously, the analysis of the *in vivo* effects is restricted to OECs and SCs from large infrahuman animals and humans (Tables 2, 3).

In vitro proliferation and mitogens for OECs and SCs from different species

The comparative analysis of OECs and SCs from different species (Table 1) is complicated for several reasons. First, so far the number of published studies in large animals and humans is very low. Although the genetic diversity of large animals and humans asks for an increased number of experiments compared to the rodent system, the opposite is in fact the case. Second, the criteria used in the present review have not been addressed systematically for OECs and SCs. Based on the assumption that human cells will behave similarly to their rodent counterpart, many investigators transferred cell culture protocols established in rodents to large animals directly (Hanemann et al., 1998; Barnett et al., 2000; Imaizumi et al., 2000; Kato et al., 2000) without testing the relevance of single culture parameters separately.

One consistent parameter across all studies is the responsiveness of OECs and SCs to typical Schwann cell mitogens (Table 1). These factors including neuregulins and fibroblast growth factor-2 were originally identified during the isolation of neonatal rodent SCs (Raff et al., 1978; Lemke and Brockes, 1984) and have been subsequently shown to stimulate proliferation of rodent, canine, porcine, primate, and human OECs (Table 1; Chuah and Teague, 1999; Wewetzer et al., 2001; Yan et al., 2001, Krudewig et al., 2006; Techangamsuwan et al., 2008, 2009; Rubio et al., 2008; Barnett et al., 2000) in addition to SCs (Table 1; Lemke and Brockes, 1984; Porter et al., 1986; Davis and Stroobant, 1990; Schubert, 1992; Rutkowski et al., 1995; Avellana-Adalid et al., 1998; Pauls et al., 2004; Monje et al., 2006; Techangamsuwan et al., 2008, 2009) independent of the developmental stage of the used cells. Although adult cells generally display lower proliferation rates than early postnatal cells, the response is apparently the same at any postnatal age. Bianco et al. (2004) reported mitogenic effects of neurotrophin-3 (NT-3) on human OECs. Further studies are needed to

Table 3
Transplantation of large animal (monkey) and human Schwann cells (SCs) into either rodent or large animals/humans. Shaded in grey are studies that used autologous transplantation of SCs. * = studies that tested OECs and Schwann cells in parallel.

Lesion model	Host	Graft	Transplant	Source	Morphological analysis	Functional analysis	References
Spinal cord demyelination (*shiverer mutant*)	Mice	Monkey	SC purified	Sural/sciatic nerve	Increased remyelination (Po)	Not performed	Avellana-Adelid et al. (1998)
Spinal cord demyelination	Rat	Human	SC purified (OEC)	OB	Increased remyelination	Increased conduction velocity	Imaizumi et al. (2000)*
Spinal cord demyelination	Rat	Human	SC purified	Sural nerve	Increased remyelination	Increased conduction velocity	Kohama et al. (2001)
Spinal cord demyelination	Mice	Monkey	SC purified	Sural nerve	Increased Proliferation/ differentiation of SC and oligodendrocytes	Increased functional recovery (Rotarod, gridwalk)	Girard et al. (2005)
Spinal cord demyelination	Mice	Monkey	SC purified	Sural nerve	Increased migration/ myelination	Not performed	Bachelin et al. (2009)
Spinal cord demyelination	Monkey (Mice)	Monkey	SC purified	Sural nerve	Increased remyelination (Po)	Increased functional recovery (Rotarod, mice)	Bachelin et al. (2005)
Nerve root transection	Monkey	Monkey	SC purified	Sural nerve	Regeneration	Muscle reinnervation	Calancie et al. (2009)
Spinal cord injury (SCI)	Human ($n = 4$)	Human	SC purified	Sural nerve	No adverse effects	Motor and sensory improvement (1/4)	Saberi et al. (2008)

demonstrate whether this neurotrophin has the same effects on SCs and whether this applies to cells from other species. Summarized, there is no evidence that the so far used growth factors affect OECs and SCs from various species differently.

The observation that OECs and SCs from different species proliferate in response to the same mitogens does not provide information about proliferation in the absence of exogenous factors. It is well established that neonatal and adult rodent OECs (Pollock et al., 1999; Alexander et al., 2002; Wewetzer et al., 2001; Yan et al., 2001) and SCs (Raff et al., 1978; Wood and Bunge, 1975; Davis and Stroobant, 1990) are mitogenically quiescent after isolation and require the presence of growth factors or neurons for cellular proliferation. Although this important cellular capacity has not directly been addressed and is not readily apparent from the reports published so far, there is growing evidence that large animal and human OECs and SCs behave differently. Recently, Rubio et al. (2008) demonstrated that rodent but not primate OECs enter a senescent state rapidly *in vitro*. Previously, we reported similar properties for canine OECs. Even under serum-free culture conditions, canine OECs and SCs proliferated over many passages (Techangamsuwan et al., 2008). Interestingly, no differences were found between canine OECs and SCs (Techangamsuwan et al., 2008). The idea that proliferation in OECs and SCs is controlled by similar mechanisms is also underscored by the recent unexpected finding that transfection at early passage with human TERT significantly reduced proliferation in both cell types to the same extent (Techangamsuwan et al., 2009). Proliferation of porcine OECs also does not require addition of growth factors (Radtke et al., 2004) and cells can be grown for a limited time *in vitro* in the absence of mitogen stimulation (Radtke et al., 2010b). Whether human OECs or SCs share the capacity of autonomous growth *in vitro* with primate and canine glia is not clear. Since many studies focused on the effects of growth factors and investigated proliferation in the absence of growth factors, the data can be only indirectly discerned from the reports. Such an analysis, however, demonstrates that human OECs and SCs display a limited growth factor-independent proliferative capacity (Moretto et al., 1984; Van den Berg et al., 1995).

Another crucial aspect for the comparative analysis is the glial cell response to increased intracellular cAMP levels. It has been recognized for some time, that proliferation of rodent SCs and OECs is stimulated by agents increasing the intracellular cAMP level, e.g. forskolin, dibutyryl-cAMP, cholera toxin (Raff et al., 1978; Sobue et al., 1986; Davis and Stroobant, 1990; Chen et al., 1991; Stewart et al., 1991; Jessen et al., 1991). The majority of the studies agree that these agents under serum-containing culture conditions are sufficient to drive proliferation, while absence of serum requires the addition of growth factors. In the presence of serum pharmacologically induced elevations in intracellular cAMP results in synergistic effects on proliferation, which is paralleled by a transition in the morphology from a spindle-shaped to a more flattened phenotype (Jessen et al., 1991; Morgan et al., 1991). The cells then display a fenestrated cytoplasm and are difficult to differentiate from fibroblast-like cells under phase contrast optics. The same has been shown for rodent OECs (Wewetzer et al., 2001; Yan et al., 2001).

When it comes to human and large animal glia, the picture is not as clear. This is due, as outlined above, to the close orientation of human studies on the rodent model, resulting in routine applications of a forskolin/growth factor mixtures rather than an independent analysis of the forskolin effect alone (Imaizumi et al., 2000, Smith et al., 2002; Kato et al., 2000). The majority of studies reported that forskolin potentiated the effects of growth factors in human glia but had no effects when applied alone (Levi et al., 1995; Rutkowski et al., 1995; Casella et al., 2000; Monje et al., 2006). This is underscored by findings of Avellana-Adalid et al. (1998) in the monkey, where forskolin stimulates proliferation of SCs only in combination with growth factors. Forskolin in human SCs was shown to increase neuregulin receptors without increasing receptor mRNA in a density-dependent manner (Casella et al., 2000; Fregien et al., 2004). However, other *in vitro* studies failed to detect any effects of forskolin on human OEC and SC proliferation, even when applied in combination with other growth factors (Morrissey et al., 1995; Barnett et al., 2000).

Analysis of the morphological phenotype of forskolin-treated human glia, although not explicitly addressed in these studies, clearly reveals that human SCs do not alter their morphological phenotype in response to forskolin (Levi et al., 1995; Hanemann et al., 1998). This is in striking contrast to the rodent model. Interestingly, both canine and porcine OECs and SCs treated with forskolin neither increased proliferation nor altered their morphology as observed in rats (Krudewig et al., 2006; Techangamsuwan et al., 2008; 2009). Since forskolin effectively stimulates intracellular cAMP level in a variety of different canine cell types (Luo et al., 1997; Zhang et al., 2000; Chu et al., 2004), it has to be concluded that the intracellular signalling pathways downstream to this event are different between rodent and humans, and, that canine cells are closely related to human cells.

Whether there is a significant difference in cAMP signalling between OECs and SCs in large animals or humans remains undetermined due to the low number of studies focusing on the effect

of forskolin in humans and primates. This hinders the critical comparison between both cell types. At present, there is no indication that OECs behave differently from SCs. With regard to the canine system, direct comparison between OECs and SCs from the same species did not reveal any significant differences (Techangamsuwan et al., 2008; 2009). Taken together it can be concluded from these data, that large animal and human OECs and SCs do not display the same complex response to increased intracellular cAMP glia as observed in rodent glia. In this respect, the canine system may be a relevant translational model (Krudewig et al., 2006; Techangamsuwan et al., 2008).

Growth, differentiation, and immortalization of OECs and SCs from different species in long-term culture

The question how cellular proliferation is controlled is not only relevant for the expansion of cells for transplantation purposes but also for estimating the risk of immortalization and tumor formation following mitogen stimulation and implantation into the nervous system (Langford et al., 1988; Emery et al., 1999). What is also important is the antigenic expression because a differential regenerative capacity of a specific cell type is only conceivable within a distinct gene expression profile. Although several groups carried out systematic molecular studies (Vincent et al., 2005; Boyd et al., 2006; Franssen et al., 2008), the number of differentially expressed candidate genes is still low. Only a limited number of studies have focused on the behaviour of OECs and SCs in long-term culture and the question of whether these cells have the potential to undergo immortalization. However, the comparative analysis of this question is of particular relevance and reveals significant differences between the species that may be relevant to cell transplantation. As described above, rodent glia does not proliferate in the absence of growth factors. It is well known that sustained mitogen stimulation either by forskolin alone or in combination with growth factors is sufficient to induce immortalization in rodent OECs (Sonigra et al., 1996) and SCs (Porter et al., 1987; Eccleston et al., 1991; Goda et al., 1991; Bolin et al., 1992; Watabe et al., 1995; Shen et al., 2002; Saravanan et al., 2007). Thus, there seems to be a reciprocal relation between autonomous growth *in vitro* and the susceptibility to immortalization.

Long-term studies have been carried out with primate OECs (Rubio et al., 2008) and canine SCs and OECs (Krudewig et al., 2006; Techangamsuwan et al., 2008; 2009). Cells could be easily maintained over many passages but immortalization was not observed. Similarly, long-term *in vitro* studies in humans lacked features of immortalization (Moretto et al., 1984; van den Berg et al., 1995; Rubio et al., 2008). How porcine glia fits into this categorization is not yet clear. Recently, we noted that the proliferation rate of porcine OECs declines during a six week culture interval (Radtke et al., 2010b). As outlined below, this reduced proliferative capacity correlated with a reduced remyelinating potential following transplantion into the demyelinated spinal cord (Radtke et al., 2010b).

Although there have been several molecular studies on the specific gene expression of rodent OECs and SCs (Vincent et al., 2005; Boyd et al., 2006; Franssen et al., 2008), there are only a few data available on putative differences in the expression between species. Although expression of classical cell type-specific markers, such as the neurotrophin receptor p75 ($p75^{NTR}$), seems to be well conserved across the species, differences with respect to the time course of expression have been reported recently. Parallel to differences in the proliferation there is distinct regulation of $p75^{NTR}$ expression in rodent and porcine and primate OECs (Rubio et al., 2008). While primate OECs display stable expression of $p75^{NTR}$ *in vitro* (Rubio et al., 2008) both rodent and porcine OECs display down-regulation *in vitro* during long-term cultivation (Rubio et al., 2008; Radtke et al., 2004, 2010b) OECs and SCs from adult dogs have been shown to behave similar to primate cells (Krudewig et al., 2006; Techangamsuwan et al., 2008, 2009) and expression of $p75^{NTR}$ is maintained throughout multiple passages.

Taken together, it is concluded that significant species-specific differences exist regarding proliferation and that canine OECs and to a lesser extent porcine OECs and SCs share a number of characteristics with human cells. These data suggest that canine, primate and human but not rodent and porcine OECs and SCs can easily maintained in long-term culture. Contrary to rodent cells, however, there is no immortalization of the cells. This indicates subtle differences in the growth control of glia between the different species. Mitogen stimulation can be ruled out as the only critical parameter, since canine glia does not immortalize even under sustained mitogen stimulation. The sustained proliferative capacity of canine, primate and human OECs and SCs is associated with the stable expression of $p75^{NTR}$ (Rubio et al., 2008; Techangamsuwan et al., 2008; 2009). Again, this seems to be in contrast with the rodent system. As shown by Rubio et al. (2008) rodent but not primate OECs displayed down-regulation of the neurotrophin receptor in long-term culture.

Transplantation and in vivo effects OECs and SCs from different species

Although there is a huge number of *in vivo* studies demonstrating that OECs and SCs promote axonal regrowth and remyelination (Wewetzer et al., 2002; Santos-Benito and Ramón-Cueto, 2003), the specific regenerative potential of both cell types even in the rodent model is still not clearly defined (Radtke and Wewetzer, 2009). The *in vivo* paradigms used to define the *in vivo* effects of OECs in the rat originally included dorsal horn rhizotomy, corticospinal tract or spinal cord transection in addition to ethidium bromide-induced demyelination (Ramón-Cueto and Nieto-Sampedro, 1994; Li et al., 1997; Navarro et al., 1999; Kato et al. 2000). While the capacity of OECs to promote axonal ingrowth into the CNS after rhizotomy has been questioned recently (Goméz et al., 2003; Ramer et al., 2004; Riddell et al., 2004), there is also a controversy about the myelinogenic potential of transplanted OECs (Harvey and Plant, 2006; Wewetzer and Brandes, 2006). Due to the lack of cell type-specific markers for OECs and SCs, it cannot be excluded presently that presence of peripheral type myelin after transplantation of OECs is at least in part due to SCs contaminating the OEC transplants. Classical studies in the rodent model focused on axonal regeneration in the spinal cord and reported that OECs and SCs increased long distance axonal growth and axonal sprouting at the lesion site, respectively (Li and Raisman, 1994; Li et al., 1997; Ramón-Cueto et al., 2000; Imaizumi et al., 2000). Due to the lack of comparable approaches testing both cell types in parallel in the same model, the relevance of this effect remains to be confirmed (Wewetzer et al., 2002).

The discussion of these data aims to provide a historical background rather than to concentrate on discrepancies to the rodent model. The relevant questions within the scope of the present review are whether OECs and SCs from large animals display similar or distinct *in vivo* effects compared rodent glia, and, mechanistically and easier to answer, which experimental models have been chosen to demonstrate the *in vivo* capacity of large animal and human OECs. To allow a focussed interpretation, only studies that used large animal and human glia for grafting were included in the present analysis. The fact that OECs (Table 2; Barnett et al., 2000; Imaizumi et al., 2000; Kato et al., 2000, Smith et al., 2002; Deng et al., 2006; Shyu et al., 2008) were more frequently used for transplantation than SCs (Table 3; Avellana-Adalid et al., 1998; Imaizumi et al., 2000; Kohama et al., 2001; Girard et al., 2005; Bachelin et al., 2010) most likely reflects the idea that the regenerative potential of these cells is superior to that of SCs. Surprisingly, the vast majority of *in vivo* studies with OECs and SCs and rodent recipients (Tables 2, 3, white) used chemically induced demyelination as experimental approach (e.g. Bachelin et al., 2005; Girard et al., 2005; Kohama et al., 2001) instead of spinal cord

transection or contusion injury (Deng et al., 2006; Gorrie et al. 2010). This indicates that the classical injury models that mainly defined the regenerative capacity of rodent OECs were only rarely used for large animal and human OECs. One of the few studies that critically compared the effects of rat and human OECs after implantation into the intact and lesioned rat spinal cord was carried out by Deng et al. (2006). However, since the authors mainly concentrated on the survival and migration of the grafted cells, this study does not provide any clue to answer the question whether large animal as well as human OECs and SCs possess the same regenerative potential as rodent cells. Guest et al. (2008) who used spinal cord transection and transplantation of human OECs failed to detect long tract corticospinal tract regeneration, while Calancie et al. (2009) reported on axonal regeneration after transplantation of human SCs into mice. It also becomes evident that the majority of studies demonstrated increased remyelination by OECs from large animals in comparison to vehicle controls morphologically while functional analyses were often included only in studies using SCs (Imaizumi et al., 2000; Kohama et al., 2001; Girard et al., 2005). This observation further underscores the need to analyze effects of the transplanted glial cells in the same experimental paradigm (Wewetzer et al., 2002; Radtke and Wewetzer, 2009).

It can be concluded that transplantation of large animal and human OECs in the rodent model apparently increased the formation of morphologically detectable myelin. Whether this correlates with a functional improvement or one of the two cell types displays a superior regenerative potential remains unclear at present. What also remains to be established is whether human and rodent OECs and SCs differ in their *in vivo* actions from each other. The result of the present analysis is sobering since the regenerative potential of large animal and human OECs and SCs is still poorly defined. In this respect, the autologous transplantation studies do not provide more information (Tables 2, 3). Since the main emphasis of these studies is the detection of putative adverse effects, which were either not (Saberi et al., 2008) or only rarely observed (Lima et al., 2006), no conclusions regarding the regenerative potential of the implanted cells can be drawn. The question is whether large animal models may provide valuable information of the *in vivo* effects and help to improve human procedures. Jeffery et al. (2005) reported that autologous transplantation of canine OECs is reliable and safe in naturally occurring spinal cord lesion models. It is the hope that such studies in translational models will speed up the transfer of experimental data to clinical practice (Jeffery et al., 2006).

Conclusions

In the present review a comprehensive *in vitro* and *in vivo* analysis of OEC and SC properties based on own and published experiments was performed. The presented evidence clearly reveals the existence of species-specific properties of growth-promoting glia. Based on the *in vitro* analysis of cellular proliferation, we conclude that human and rodent glia display significant differences in the control of cellular proliferation and that glia from large animals, e.g. dogs, pigs and monkeys more closely resemble their human counterpart. Moreover, the summarized data are also evidence for a close relationship between OECs and SCs. There were no significant differences between both cell types that became evident during this analysis.

However, whether the observed differences are relevant for the *in vivo* application of the cells is not apparent from the currently available data. The *in vivo* effects of OECs and SCs from large animals and humans are still poorly defined. Moreover, due to the low number of comparative studies between OECs and SCs and between glia from different species, it remains to be determined whether OECs from humans share the same regenerative effects as detected in the rodent system. It is recommended to cross the bridge between humans and rodents by intensifying the work with translational models that display higher homology to the human system than rodents. In this context the canine and the porcine system may be in the future of great relevance. It is hoped that research in these translational large animal models will accelerate the transfer of experimental cell transplantation therapies to clinical practice.

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