Immunochemical and molecular characterization of a novel cell line derived from the brain of *Trachinotus blochii* (Teleostei, Perciformes): A fish cell line with oligodendrocyte progenitor cell and tanyctye characteristics

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**A B S T R A C T**

Ependymal radial glial cells, also called tanyocytes, are the predominant glial fibrillary acidic protein (GFAP)- and vimentin (VIM)-expressing cells in fish ependyma. Radial glial cells have been proposed to be neural progenitor and stem cells but their molecular expression is not well understood. Previous studies revealed that fish neural progenitor and neural stem cells have A2B5, a marker for oligodendrocyte progenitor cells (OPCs). In this study, an A2B5+ cell line, SPB, was isolated from the brain of the teleost *Trachinotus blochii* and characterized. SPB cells usually grew as polygonal epithelial cells, but at high density, long processes were commonly observed. Using immunocytochemistry, SPB cells were shown to exhibit oligodendrocyte markers such as galactocerebroside and Olig2, and radial glial cell markers such as brain lipid-binding protein, GFAP, Sox2, and VIM. SPB cells were also observed to have DARPP-32, a marker for tanyocytes in mammals, and primary cilia. RT-PCR additionally revealed expression of bone morphogenetic protein 4, connexin35, Noggin2, and proteolipid protein in SPB cells. Results of this study suggest that SPB cells are OPCs that can display tanyctye characteristics. Fish tanyocytes can be neural stem cells suggesting that SPB cells are neural stem cells. SPB is the first fish cell line showing primary cilia and markers for both OPCs and tanyocytes.

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1. Introduction

In vitro studies have shown the presence of oligodendrocyte progenitor cell (OPC) markers A2B5, proteolipid protein (PLP), and DM20, as well as the presence of the astroglia marker, glial fibrillary acidic protein (GFAP), in teleost brain cells (Jeserich and Stratmann, 1992; Sivron et al., 1992, 1994; Wen et al., 2008a, 2009). Most of the proliferating cells in adult fish brain localize along the ependyma where neural stem and neural progenitor cells exist (Zupanc and Horschke, 1995; Ekström et al., 2001; Adolf et al., 2006; Chapouton et al., 2006; Grandel et al., 2006; Pellegrini et al., 2007; Lam et al., 2009). Ependymal radial glial cells or tanyocytes are the most abundant GFAP- and vimentin (VIM)-expressing cells in the central nervous system (CNS) of adult teleosts (Kálmán, 1998; Arochena et al., 2004; Lazzari and Franceschini, 2004) suggesting that GFAP-expressing cells cultured in vitro are tanyocytes.

Fish tanyocytes have been reported to express aromatase B (AroB), brain lipid-binding protein (BLBP), NADPH-diaphorase, glutamine synthetase (GS), Nestin, S100 protein, Sox2, and VIM in addition to GFAP (Forlano et al., 2001; Adolf et al., 2006; Germanà et al., 2008; Lam et al., 2009; Tong et al., 2009; Wen et al., 2009). Fish tanyocytes are similar to other ependymal cells, and exhibit epithelial characteristics, including the presence of keratins (8 and 18), desmosomes, and connexin-43 (Cx43) type gap junctions (Bodega et al., 1993, 1994, 1995; Bruni, 1998; Hernández et al., 1999, 2009; Wen et al., 2009); moreover, they may or may not be ciliated (Ma, 1993; Shiota et al., 1997; Bruni, 1998).

In rodents, four OPC types have been observed: gial-restricted precursors, motor neuron–oligodendrocyte precursors, oligodendrocyte-type 2 astrocyte (O-2A) progenitors, and polydendrocytes (Liu and Rao, 2004). Typically, O-2A cells arise from A2B5-positive, platelet-derived growth factor α receptor (PDGFRα)-negative gial-restricted precursors, but some O-2A cells are derived from A2B5-negative and PDGFRα- and NG2 (AN2)-positive polydendrocytes (Baracksky et al., 2007). Gial-restricted precursors are derived from neuroepithelial cells (Rao and Mayer-Proschel, 1997) whereas polydendrocytes are generated from GFAP-, VIM-, or PDGFRα-positive type B cells (Menn et al., 2006; Li and Grumet, 2007), which are generated from radial glial cells (Merkle et al., 2004). OPCs have been reported to exhibit the stem cell markers Nestin and Sox2, and may also exhibit oligodendrogenic...
transcription factors Olig1, Olig2, and Nkx2.2 (Liu et al., 2002; Liu and Rao, 2004; Jakovcevski et al., 2009). During oligodendrocyte maturation, the OPCs may also exhibit the oligodendrocyte precursor proteins O1 and O4, PLP, DM20, Sox8, Sox9, Sox10, galactocerebroside (GalC) and/or myelin basic protein; however, they may show a loss of Sox2, NG2, A2B5, and PDGFβR expression (Liu et al., 2002; Jakovcevski et al., 2009).

In the teleost CNS, a subset of radial glial cells are either OPCs or may generate OPCs (Park and Appel, 2003; Park et al., 2007; Kim et al., 2008a,b). As in rodents, fish OPCs also express oligodendrocyte transcription factors Nkx2.2, Olig1, Olig2, and Sox10 (Kucenas et al., 2008; Schebesta and Serluca, 2009; Zammnio and Appel, 2009). The presence of primary cilia, along with expression of DARPP-32 and anti-Olig2, have been described (Wen et al., 2008a, 2009). The anti-DARRP-32 and anti-Olig2 antibodies were predicted cross-reactive with fish antibody against the amino acid sequences of the antibodies. Secondary antibodies were FITC-conjugated anti-rabbit IgG (1:100), anti-mouse IgG (1:100), and anti-mouse IgM (1:50), and Alexa Fluor® 568-conjugated anti-rabbit IgG (1:200).

SPB cells were examined for the expression of certain molecules at selected passages. Cells (2 or 4 × 10⁶) were plated on 12-mm diameter uncoated coverslips (Glaswarenfabrik, Sondheim, Germany) in 4-well plates (Nunc). Cells were grown on coverslips at 25 °C for 1–3 days, fixed in formaldehyde (3.7% in PBS, v/v) for 10 min at room temperature, washed several times in 1% Triton X-100 PBS and then incubated with the primary antibody at 37 °C for 1 h. After several washes, cells were incubated with the appropriate secondary antibody at 37 °C for 30 min. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI, Sigma) for 1 min. Labeled cells were visualized under an Olympus IX51 inverted fluorescence microscope (Yuan Li instrument Co., Taipei, Taiwan) or an Axiovert 200 fluorescence microscope (Carl Zeiss, Göttingen, Germany). Negative controls (omission of the primary antibody) were included in each experiment. The primary antibody, target, source, and dilution for immunocytochemistry are listed in Table 1. In this study, an A2B5-expressing continuous cell line (SPB) was established from the brain of snubnose pompano (Trachinotus blochii) and was analyzed using immunocytochemistry with a panel of antibodies and RT-PCR to reveal molecular expressions. Our results demonstrate that SPB cells have both tanycyte- and OPC-specific characteristics.

2.2. Immunocytochemistry

Fish neural cells are demonstrated expressing antigens that are conserved in mammalian neural cells and can be identified using the antibodies against mammalian antigens. The primary antibodies, targets, and dilutions for immunocytochemistry are listed in Table 1. Total RNA in SPB cells at passages 40–60 was isolated for RT-PCR using the blood/culture cell total RNA isolation kit (Favorgen, Ping-Tung, Taiwan) according to the manufacturer’s protocols. RNA concentration and purity were determined by measuring the absorbance at 260 nm and 280 nm. RNA (5 μg or 5 μL per sample) was used to generate cDNA using the MMLV-RT kit (Promega, Madison, WI, USA). Primer sets used in this study are listed in Table 2. Primers other than those previously reported were designed using Primer Premier 5 software (Premier Biosoft International, Palo Alto, CA, USA). Primer sets specific for BLBP, Noggin2, and Olig2 were designed, respectively, from spotted green pufferfish (Tetraodon nigroviridis CR684531), three-spined stickleback (Gasterosteus aculeatus BT028450), and zebrafish (Danio rerio).
AF442964). PCR amplifications were performed using the Taq DNA Pol Master Mix (Ampliqon, Copenhagen, Denmark). cDNA (1 µL), 1 µM each of forward and reverse primers, and 12.5 µL of Master Mix were added to each 0.2-mL thin-wall PCR tube according to the manufacturer’s protocol. PCR was carried out under the following conditions: 30 cycles of denaturation at 95 °C for 1 min, annealing (as in Table 2) for 30 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 7 min. PCR products were electrophoresed on a 1.5% agarose gel. The observed sizes corresponded to the predicted values (Table 2) were cut from the agarose and then purified with Gel purification kit (Favorgen) according to the manufacturer’s protocols. Sequencing of the DNA fragments was performed commercially (Genomic BioSci & Tech, Taipei, Taiwan). Sequences were identified using BLASTN (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

3. Results

3.1. Establishment of an A2B5-expressing cell line

Four weeks after the start of primary culture, a confluent monolayer was obtained. Most of the cells that migrated and proliferated in the culture were polygonal epithelial-like cells (Fig. 1). Oligodendrocyte-like cells, which grew on, or at, the margins of the monolayer, were also frequently observed (Fig. 1, arrows). Epithelial-like cells remained predominant for several passages (data not shown). Immunocytochemistry at the third passage showed that many of the cells expressed keratin in the cytoplasm and A2B5 on the surface (Fig. 2). The A2B5-expressing cells at the third passage were isolated by resuspending the cells and then purifying them with monoclonal mouse anti-A2B5 and rat anti-mouse IgM immunomagnetic beads. Subsequently, the purified cells attached and grew on the culture flask, and over 95% of the cells had magnetic beads on their surface. The beads were detached from the cells upon treatment with trypsin and subsequent subculturing. Most (75–90%) of the purified cells, dependent on cell density, displayed a polygonal morphology, and a few cells (10–25%) had one or more long processes. The purified cells that expressed varying levels of A2B5 were subcultured for more than 50 times and were designated as the SPB continuous cell line. Cells of the established SPB line exhibited morphological and molecular expression patterns similar to those observed in the early passages of A2B5-expressing cells.

3.2. Immunocytochemical characterization

For immunocytochemistry, SPB cells at every 5–8 passages (at 5–60 passages) were grown on uncoated coverslips. To characterize expression of oligodendrocyte markers, SPB cells were stained with

Table 2

<table>
<thead>
<tr>
<th>Primer set</th>
<th>Sequences (5′–3′)*</th>
<th>Product size (bp)</th>
<th>Annealing (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLBP</td>
<td>F: atggtcgacgcctctgtgc R: ggccttcatgccttctcgta</td>
<td>405</td>
<td>57</td>
<td>This study</td>
</tr>
<tr>
<td>BMP4</td>
<td>F: gtttaacctcagacagctc R: agcttctcacttacatccct</td>
<td>782</td>
<td>55</td>
<td>Wen et al. (2009)</td>
</tr>
<tr>
<td>Cx35</td>
<td>F: wgktgktggtgattgtcgc R: tggctctctgtggctcgcgt</td>
<td>619</td>
<td>50</td>
<td>Wen et al. (2008a)</td>
</tr>
<tr>
<td>Cx43</td>
<td>F: ggctgctcatccacagtgc R: gactgctctacagcgtcggg</td>
<td>325</td>
<td>57</td>
<td>Wen et al. (2008a)</td>
</tr>
<tr>
<td>GFAP-3</td>
<td>F: agaactctgcaatgctcaag R: ctcctgactctctctactgat</td>
<td>230</td>
<td>55</td>
<td>Wen et al. (2009)</td>
</tr>
<tr>
<td>GS</td>
<td>F: aagggctctcaacacgtcactc R: cagccagcaccgttccagtt</td>
<td>545</td>
<td>55</td>
<td>Wen et al. (2008a)</td>
</tr>
<tr>
<td>Noggin2</td>
<td>F: atgggccttcaaacacgtcactc R: cagccagcaccgttccagtt</td>
<td>634</td>
<td>57</td>
<td>This study</td>
</tr>
<tr>
<td>Olig2</td>
<td>F: tggctctmaaagtctacacga R: cagccagcaccgttccagtt</td>
<td>247</td>
<td>57</td>
<td>This study</td>
</tr>
<tr>
<td>PDCRF5x</td>
<td>F: aagggctctcaacacgtcactc R: cagccagcaccgttccagtt</td>
<td>735</td>
<td>55</td>
<td>Wen et al. (2009)</td>
</tr>
<tr>
<td>PLP</td>
<td>F: ctaattgctccgtcagg R: cagccagcaccgttccagtt</td>
<td>344</td>
<td>50</td>
<td>Wen et al. (2008a)</td>
</tr>
<tr>
<td>Sox2</td>
<td>F: tgcggctctccgtgttgagta R: ggccttcatgccttctcgta</td>
<td>941</td>
<td>55</td>
<td>Wen et al. (2009)</td>
</tr>
</tbody>
</table>

* F: forward; R: reverse; degenerate bases, k: g or t; m: a or c; r: a or g; s: c or g; w: a or t.
rabbit anti-GalC and anti-Olig2. All cells were labeled by the antibodies, but the intensity of the labeling varied among cells. GalC staining was especially strong in oligodendrocyte-like cells (Fig. 3). The staining with anti-Olig2 appeared mainly in the nucleus and nucleolus; however, Olig2 in the cytoplasm was also noted (Fig. 4). The Olig2-expression cells also showed GFAP in the cytoplasm (Fig. 4).

Since fish OPCs in vitro have characteristics of tanyocytes (Wen et al., 2008a, 2009), the SPB cells were examined for the presence of tanyocyte markers, including the presence of a primary cilium and expression of BLBP, Cx43, DARPP-32, GFAP, GS, keratin, S100, Sox2, and VIM. Both polygonal and process-bearing SPB cells expressed these proteins (Fig. 5A–H). SPB cells have a primary cilium (Fig. 5A, F) as determined by anti-acetylated α-tubulin staining (Alieva et al., 1999). DARPP-32 labeling in SPB cells was high in the nucleus and scarce in the cytoplasm (Fig. 5A, D). Similar to the DARPP-32 results, anti-BLBP labeling was concentrated in the nucleus of SPB cells and was scarce in the cytoplasm (Fig. 5B). Bundles of GFAP were observed in the cytoplasm in radial glia-like SPB cells, but GFAP labeling was faint in polygonal SPB cells (Fig. 5B). The staining with anti-VIM (Fig. 5C, D, G) was similar to that of GFAP, but was more intense, while the staining with anti-Sox2 was approximately homogenous in the nucleus and cytoplasm (Fig. 5C, E). However, the Sox2- and BLBP-positive polygonal cells present in SPB cells and in previous studies (Wen et al., 2008a, 2009), showed sparse GFAP (Fig. 5B) and VIM (Fig. 5D) labeling. Keratin was observed in the cytoplasm of the polygonal cells (Fig. 5E) and in those cells with long processes, but the polygonal cells had higher levels (data not shown). The staining for the gap junction protein Cx43 was punctate, and either

adjoint to, or disconnected from, the polygonal cells (Fig. 5F, G), but Cx43 staining was scarce in the SPB cells with long processes (Fig. 5G). Except for a few oligodendrocyte-like cells that exhibited high levels of GS and S100, staining for these proteins was sparse in most SPB cells (Fig. 5H).

3.3. Specific gene expression

Purified DNA fragments from the SPB cell line used for sequencing were confirmed by ensuring that their sizes corresponded to the predicted values (Fig. 6). All of the SPB nucleotide sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html; BLBP GU001777, BMP4 GU001778, Cx35 GU001779, Cx43 GU001780, GFAP GU001781, GS GU001782, Noggin2 GU001783, Olig2 GU001784, PDGFRα GU001785, PLP GU001786, and Sox2 GU001787). The BLAST program revealed that the nucleotide sequences encoding the BMP4, Cx35, Cx43, GFAP, GS, PDGFRα, PLP and Sox2 proteins of SPB respectively had 93, 96, 100, 100, 93, 89, 94, and 96% identities with the respective BMP4 (FJ436409), Cx35 (EU798295), Cx43 (EU798290), GFAP (EU798291), GS (EU798292), PDGFRα (EU798293), PLP (EU798294), and Sox2 (FJ426995) proteins of GBC4 (orange-spotted grouper E. coioides, data not shown). Respectively, the SPB BLBP, Noggin2, and Olig2 nucleotide sequences had 91, 93, and 85% identities with pufferfish (T. nigroviridis) BLBP (CR684531) and Noggin2 (AY779056), and with zebrafish (D. rerio) Olig2 (AF422964) (data not shown). These data confirm that SPB cells express protein molecules characteristic of both OPCs and tanyocytes.

4. Discussion

Here, an A2B5-expressing SPB cell line from the brain of snubnose pompano was established and characterized. SPB exhibited markers of oligodendrocytes and astroglia similar to those reported in GBC4, a tanyocyte-like cell line (Wen et al., 2009). In addition, SPB cells expressed Olig2 and Noggin2. Moreover, SPB cells have a single primary cilium that has not been observed in GBC4 cells. These differences indicate the presence of heterogeneity in fish OPCs and tanyocytes. Our results suggest that SPB cells are tanyocyte-like neural stem cells with more OPC characteristics than those in GBC4 cells. Several studies have reported the cultivation of neural progenitor and neural stem cells from adult fish brain (Hinsch and Zupanc, 2006; Wen et al., 2008a,b; Servili et al., 2009). Many fish neural progenitor and neural stem cells show neuroepithelial characteristics and exhibit mature neural
markers in vitro. A review of the results of those studies suggests the presence of heterogeneous neural progenitor and neural stem cells among these teleost cultures. However, due to a lack of type-specific markers, the relationship between neural progenitor and neural stem cells is unclear.

4.1. Molecular expression of fish OPCs in vitro

Fish OPCs in vitro are characterized by the expression of A2B5. They are either fibroblast-like or epithelial-like, and may or may not have GFAP. In this study, SPB cells have been shown to not only express OPC markers A2B5, PLP, PDGFRα, and Sox2, a stem cell marker, but also exhibit markers for astroglia and neurons as has been previously reported in TB2, a tilapia (Oreochromis mossambicus and O. niloticus hybrid) cell line, and in GBC4 cells from grouper (Wen et al., 2008a, 2009). Fish OPCs may generate from radial glial cells or be a subtype of radial glial cells; therefore, the detection of astroglia markers in the cells was not surprising. SPB and GBC4 cells show a greater degree of epithelial characteristics than that in TB2 cells (Wen et al., 2008a). Moreover, SPB cells express Olig2 and Noggin2, whereas GBC4 cells do not (Wen et al., 2009). GBC4 and TB2 cells have exhibited expression of neuron marker Cx35 and tyrosine hydroxylase (TH) in previous studies (Wen et al., 2008a, 2009); however, SPB cells did not express TH (data not shown), whereas they did express Cx35.

Expression of Sox10 has been observed in OPCs in zebrafish CNS (Cunliffe and Casaccia-Bonnefil, 2006; Li et al., 2007). Here, RT-PCR examination of Sox10 expression in SPB was negative (data not shown), similar to the results reported for GBC4 cells (Wen et al., 2009). Because the Sox10 protein is needed for oligodendrocyte terminal differentiation and myelination (Stolt et al., 2002; Li et al., 2007), the results indicate the SPB cells are in an early OPC stage. Although GalC expression is restricted in mature oligodendrocytes in mammals (Liu and Rao, 2004), it does appear in SPB and other fish neural progenitor cell lines, including GBC4 and TB2 (Wen et al., 2008a, 2009). SPB also shows radial glia markers, including BLBP, GFAP, GS, S100 and Vim, although the expression of GS and S100 is sparse. Fish neural stem and progenitor cells show characteristics different from their counterparts in mammals. They retain neuroepithelial properties, including keratin, Cx43 type gap junctions, desmosome junctions, and PDGFRα expression (Wen et al., 2008a, 2009), as well as exhibiting apical–basal polarization and ventricle contact (Kaslin et al., 2009).

4.2. SPB cells exhibit specific molecules of tanyocyte

Tanyocytes comprise a heterogeneous population of specialized cells lining the ependymal layer of CNS and were classified broadly into ependymal and extraependymal cells (Horstmann, 1954). In adult mammals, tanyocytes are abundant in the third ventricle (hypothalamus) wall and have been studied extensively. On the basis of morphology, topography, and metabolism, four types of hypothalamic tanyocytes (cx1, cx2, β1, and β2) are distinguishable (Akmayev and Fidelina, 1976; Rodriguez et al., 2005). Numerous proteins, including GFAP, DARPP-32, Nestin, O4, S100, and Vim have been detected in hypothalamic tanyocytes; however, none of the proteins are uniquely present in those cells (Rodriguez et al., 2005). Tanyocytes share some features with radial glia but display several properties, including DARPP-32, keratin 8 and 18, glutamate transporters, and glucose transporters, that clearly distinguish them from subependymal radial glia.

In the adult teleost CNS, most radial glial cells have cell bodies located at the ependyma and are recognized as ependymal radial glial cells or tanyocytes. In contrast to mammals, adult fish CNS tanyocytes are a subtype of radial glial cells (Kálman, 1998). Molecular expression of tanyocytes in fish CNS is unclear, yet may be similar to that in fish radial glial cells, which exhibit AroB, BLBP, Nestin, Olig2, GS, S100, and Sox2, in addition to GFAP and Vim (Forlano et al., 2001; Menuet et al., 2003; Pellegrini et al., 2005, 2007; Ari and Kálman, 2008; Kim et al., 2008a; Lam et al., 2009; Tong et al., 2009). Fish tanyocytes, and other ependymocytes, have keratins, desmosomes, and gap junctions (Bodega et al., 1993, 1995) and may or may not exhibit cilia on the ventricular surface (Ma, 1993; Shioda et al., 1997). In this study, SPB cells are recognized as tanyocytes as they have DARPP-32, primary cilia, radial glia markers, and a long GFAP- and Vim-positive process. DARPP-32 is a specific marker for dopaminergic cells including choroidal epithelial cells, neurons, pituicytes, and tanyocytes (Meister et al., 1988), but was not detected in carp (Cyprinus carpio) (Hemmings and Greengard, 1986) and goldfish (Carassius auratus) brain (Hemmings et al., 1992). The DARPP-32 negative results in those previous studies and the positive result in this study might be due to the use of different antibodies.

4.3. Identity of SPB

Olig2-expressing radial glial cells in fish CNS have been observed to generate neurons and oligodendrocytes, but astrocyte generation has not been reported (Park et al., 2002, 2007; Reimer et al., 2008; Zannino and Appel, 2009). In mammals, the fate and morphogenesis of neural progenitor cells are controlled by BMPs and Noggin (Bani-Yaghoub and Appel, 2009). In mammals, the fate and morphogenesis of neural progenitor cells are controlled by BMPs and Noggin (Bani-Yaghoub and Appel, 2009). Fish OPCs may generate from radial glial cells or be a subtype of radial glial cells, which exhibit AroB, BLBP, Nestin, Olig2, Nestin, Olig2, S100, and VIM have been detected in hypothalamic neurons, pituicytes, and tanyocytes (Meister et al., 1988), but was not detected in carp (Cyprinus carpio) (Hemmings and Greengard, 1986) and goldfish (Carassius auratus) brain (Hemmings et al., 1992). The DARPP-32 negative results in those previous studies and the positive result in this study might be due to the use of different antibodies.

Fig. 6. RT-PCR analyses of SPB cells to detect transcripts for BLBP (405 bp), BMP4 (782 bp), Cx35 (619 bp), Cx43 (325 bp), GFAP (230 bp), GS (545 bp), Noggin2 (634 bp), Olig2 (247 b), PDGFRα (735 bp), PLP (344 bp), and Sox2 (941 bp). The amplicons having the predicted size were purified, cloned, and sequenced to verify the specificity of the PCR reaction. M: 100 bp ladder.
oligodendrocytic differentiation (Kasai et al., 2005). Additionally, Olig2 represses the generation of astrocytes from progenitor cells, and an export of Olig2 to the cytoplasm is essential for astrocyte differentiation (Setoguchi and Kondo, 2004). The role of BMPs and Noggin in the development of fish neural cells is uncertain and expression of BMP4 and Noggin2 in the Olig2-expressing cells in fish CNS needs to be studied.

SPB cells exhibit markers for OPCs as well as for tanyocytes and share some properties with type B astroglia in the subventricular zone (SVZ) of the adult mammalian brain. Type B astroglia have a single primary cilium, PDGFRα, and GFAP, are the neural stem cells in adult mammalian brain, and are heterogeneous (García-Verdugo et al., 1998; Danilov et al., 2009). SPB cells are suggested OPCs, but the ability to generate neurons is questionable.

4.4. Functions of fish tanyocytes

The function of tanyocytes remains largely speculative. Tanyocytes act as barrier cells, as do other ependymocytes, and are commonly suggested to be located between the ventricle and the neural and portal capillaries; thereby allowing uptake and transport of substances from the central spinal fluid, guiding migration and regeneration of neurons and axons, and regulating endocrine, immune, and reproductive functions (Rodríguez et al., 2005; Lechan and Fekete, 2007). By contrast, tanyocytes in fish brain, when functioning as radial glial cells, are active in neurogenesis and neuron guiding. However, additional functions of fish tanyocytes, other than neurogenesis, are unclear. Nevertheless, tanyocytes in the adult telencephalon contain NADPH-diaphorase suggesting that they can generate nitric oxide to modulate a variety of neural functions, including synaptic transmission, cerebral blood flow, and excitotoxicity (Ma, 1993). Expression of AroB associates with estrogen receptors (Muenet et al., 2003; Pellegrini et al., 2005) indicates that fish tanyocytes are regulated by endocrine hormones (Mourie et al., 2008).

4.5. Conclusion

Previously, GBC4 neural progenitor cells from adult fish brain have been reported to express fish radial glia specific markers including AroB, BLBP, GFAP, VIM, and Sox2 in vitro (Wen et al., 2009). In this study, the tanyocyte marker DARPP-32 and the OPC marker Olig2 were expressed in SPB in vitro. Also, primary cilia were noted on the apical surface of SPB cells but were not present on GBC4 cells. Although both GBC4 and SPB express BMP4, Noggin2 expression was only observed in SPB. Both Olig2 and Noggin2 promote oligodendrocyte generation; therefore, oligodendrocyte-like cells appear to be more common in SPB than in GBC4. Fish neural progenitor and neural stem cells and some of the genes responsible for the expression of these proteins have been identified; however, the microenvironmental factors that may induce neuron and oligodendrocyte generation are unknown. Revealing the conditions that generate large numbers of mature neurons and/or oligodendrocytes in vitro is a topic for future work.

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