

Review

Decoding the role of zebrafish neuroglia in CNS disease modeling

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ABSTRACT

Neuroglia, including microglia and astrocytes, is a critical component of the central nervous system (CNS) that interacts with neurons to modulate brain activity, development, metabolism and signaling pathways. Thus, a better understanding of the role of neuroglia in the brain is critical. Complementing clinical and rodent data, the zebrafish (*Danio rerio*) is rapidly becoming an important model organism to probe the role of neuroglia in brain disorders. With high genetic and physiological similarity to humans and rodents, zebrafish possess some common (shared), as well as some specific molecular biomarkers and features of neuroglia development and functioning. Studying these common and zebrafish-specific aspects of neuroglia may generate important insights into key brain mechanisms, including neurodevelopmental, neurodegenerative, neuroregenerative and neurological processes. Here, we discuss the biology of neuroglia in humans, rodents and fish, its role in various CNS functions, and further directions of translational research into the role of neuroglia in CNS disorders using zebrafish models.

1. Introduction: astrocytes and microglia

Neuroglia plays multiple roles in the central nervous system (CNS), as both macro- and microglia provide nutrition and support for neurons and modulate their signaling, development, migration and synaptic plasticity (Baumann and Pham-Dinh, 2001; Boullerne, 2016; Sun,

2011). Brain macroglia includes astrocytes, oligodendrocytes and ependymal cells (Sun, 2011). Oligodendrocytes produce myelin sheaths, provide neurons with crucial energy substrates and modulate their development, functioning and survival by releasing key neurotrophins, such as the nerve growth factor (NGF), the brain-derived neurotrophic factor (BDNF), the glial-derived growth factor (GDNF), ciliary

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Table 1

A brief summary of selected astrocytal biomarkers in vertebrate models.

Biomarkers	Selected cellular functions	References
GFAP (glial fibrillary acidic protein)	Cytoskeleton of mature astrocytes, synaptic plasticity	(Middeldorp and Hol, 2011)
GLAST, GLT-1 (glutamate transporters)	Excitatory neurotransmission (controls extracellular glutamate)	(Perego et al., 2000)
Vimentin	Astrocytal cytoskeleton and (with integrins) extracellular matrix	(Danielsson et al., 2018)
S100 β protein	Neuronal activity via the regulation of Ca ²⁺ turnover	(Brockett et al., 2018)
GS (glutamine synthetase)	Extracellular glutamate removal, balancing the excitatory/inhibitory neurotransmission	(Rose et al., 2013)
AQP4 (aquaporin 4)	Water permeability and neuroimmune functions	(Ikeshima-Kataoka, 2016)
Cx30; inCx43 (connexins)	Building astrocyte- and neuron-astrocyte interconnections	(Koulakoff et al., 2008)
Aldh1L1 (aldehyde dehydrogenase-1 family, 1)	Cell division and growth via <i>de novo</i> nucleotide biosynthesis	(Yang et al., 2011)
SPARC (secreted protein acidic/rich in cysteine)	Synaptic structure and extracellular matrix	(Strunz et al., 2019)
Hevin	Synapse scaffolding	(Allen and Eroglu, 2017)
TN-C (tenascin C)	Embryonic spinal development, maturation and differentiation of spinal astrocytes	(Karus et al., 2011)
TSP-1 (thrombospondin 1)	Synapse structure and spinal cord development	(Allen and Eroglu, 2017), (Cheng et al., 2016)
CCN3 (CYR61/CTGF/NOV)	Microglial neuroinflammation, secretion of cytokines/chemokines	(Le Dréau et al., 2010)
STAT3 (signal transducer and activator of transcription 3)	Reactive astrogliosis and glial scar formation	(Herrmann et al., 2008)
mI (myo-inositol)	Metabolic neuron-astrocyte shuttling, glutamine-glutamate turnover	(Carter et al., 2019; Xu et al., 2016)
NDRG2 (N-myc downstream regulated gene 2)	Cell cycle regulation, the blood-brain barrier (BBB) organization, glutamate clearance	(Li et al., 2019)
C3 (complement component 3)	Neuroinflammation in Alzheimer's disease (AD) via M1-microglia activation	(Lian et al., 2016)
BLBP (brain lipid-binding protein)	The fatty acids metabolism, astrocytes proliferation	(Li et al., 2018)
LOX (lysyl oxidase)	Astrocytal growth/proliferation, extracellular matrix	(Rivera and Butt, 2019)
FSTL1 (follistatin-like 1)	Inhibition of the expression of proinflammatory cytokines	(Cheng et al., 2017)
Reelin, SLIT1 (slit homolog 1), PAX6, Nkx6.1 (NK6-homebox 1)	Migration of neural stem niche progenitors in neurodevelopment and differentiation of astrocytes	(Chaboub and Deneen, 2012; Hochstim et al., 2008)
MMPs 2/9 (matrix metalloproteinases 2/9)	Activated production of proinflammatory cytokines	(Song et al., 2015)

neurotrophic factor (CNTF), leukemia inhibitory factor (LIF) and neuroregulins (Bankston et al., 2013; Byravan et al., 1994; Du and Dreyfus, 2002; Wilkins et al., 2003). Astrocytes establish dynamic networks (via gap junctions) with neurons, participating in their maturation, metabolism, synaptic signaling and the blood-brain barrier (BBB) function (Mahringer et al., 2014). They also produce a wide range of neurotrophins, chemokines and cytokines, some of which (e.g., interleukins (IL) IL-6, IL-1 β , tumor necrosis factor (TNF)- α and transforming growth factor (TGF)- β), play a key role in axonal regeneration, as astrocytes migrate to injured axons and form a 'glial scar' (Falnikar et al., 2015; Wang et al., 2016).

The two major subtypes of astrocytes include protoplasmic

astrocytes (mossy cells around the grey matter) and fibrous astrocytes (residing in the white matter to provide homeostasis (Matias et al., 2019; Sofroniew and Vinters, 2010)). Some additional, organ/tissue-specific astrocyte-like cells include cerebellar Bergman cells, retinal Muller cells, radial glia (RG), as well as cortical interlaminar and varicose projection astrocytes. In general, there are two functional subtypes of astrocytes, A1 and A2 (Miller, 2018). The A1 cells, activated by microglial cytokines (e.g., IL-1 α , TNF and C1q), exhibit prominent neurotoxic effect, accompanied by elevated astrocytal biomarkers glial fibrillary acidic protein (GFAP) and vimentin (Clarke et al., 2018) (Table 1). In contrast, the A2 astrocytes exert neuroprotection (e.g., in response to ischemia) and secrete anti-inflammatory factors BDNF, NGF and TGF- β

Table 2

A brief summary of selected molecular and genetic biomarkers of microglial functional phenotypes (M0-M2), based on (Bogie et al., 2014; Bok et al., 2018; DePaula-Silva et al., 2019; Karlstetter et al., 2014; Lively et al., 2018; Niraula et al., 2017; Periyasamy et al., 2018; Rangaraju et al., 2018; Ritzel et al., 2015; Rojo et al., 2014; Tang and Le, 2016; Tang et al., 2014; Timmerman et al., 2018; Walker and Lue, 2015).

M0 (resting)	M1 (pro-inflammatory)	M2 (anti-inflammatory)
Upregulated		
P2ry12 (purinergic receptor P2Y12)	IBA1/AIF1 (allograft inflammatory factor 1)	TGF- β , IL-4, IL-13, IL-10
Sall1 (salt-like transcription factor 1)	MHC II (Major histocompatibility complex II)	Scavenger receptors CD36, CD136, CD163
Fcrls (Fc receptor-like S)	TNF- α	CD206 (mannose receptor)
Slc2a5 (solute carrier family 2 member 5)	CXCL11, CCL19, CXCL10 and CXCL9 (C-X-C chemokine ligands)	CD209 (Alzheimer disease/AD brain-related)
Gas6 (growth arrest specific 6)	IL-1 β , IL-6, IL-8	Immunoglobulin Fc gamma receptors CD16, CD32, CD64
Cx3cr1 (C-X3-C motif chemokine receptor 1)	TSPO (translocator protein)	SPHK1 (sphingosine kinase)
	CCL2 (C-C motif ligand 2)	CCL22 (C-C motif ligand 22)
	CD14 (LPS receptor)	Cyclooxygenase-1 (COX-1)
	CD40 (AD brain-related)	SOC3 (suppressor of cytokine signaling 3)
	CD45 (leucocyte common antigen)	YM-1/ CHI3L3 (chitinase-3 like 3)
Il1r2b (interleukin 1 receptor like 2 b)	Toll-like receptors TLR-2, TLR-4	FIZZ1 (found in inflammatory zone 1)
	KLF4 (Krüppel-like factor 4)	IGF-1 (insulin-like growth factor 1)
	Ferritin (iron storage protein)	TREM2 (triggering receptor expressed on myeloid cells 2)
	iNOS (inducible NO synthase)	ARG1 (Arginase 1)
Downregulated		
CD200R (cell surface transmembrane glycoprotein receptor)	NADPH (nicotinamide adenine dinucleotide phosphate) oxidase	CD45 (leucocyte common antigen)
iNOS (inducible NO synthase)	CX3CR1 (C-X3-C motif chemokine receptor 1)	
ARG1 (Arginase 1)	CD200R (cell surface transmembrane glycoprotein receptor)	

(Li et al., 2019; Ponath et al., 2018). The A2 astrocytes are often activated via the epidermal growth factor (EGF) pathway (Chan et al., 2019) and express specific biomarkers, such as the scar-forming reactive astrocyte marker, signal transducer and activator of transcription 3 (STAT3) (Liddelow and Barres, 2017). Common A1/A2 molecular biomarkers (Table 1) include the glutamate transporters GLAST and GLT-1, the calcium-binding protein S100 β , glutamine synthetase (GS), aquaporin 4 (AQP4), connexins Cx30 and Cx43, aldehyde dehydrogenase 1 family, member L1 (Aldh1L1), and their respective genes (Matias et al., 2019).

Microglia originate from the hematopoietic progenitors during the embryogenesis and from macrophages in adult brain (Ginhoux et al., 2010), mostly residing in the grey matter (Staszewski and Hagemeyer, 2019). The main source of central pro-inflammatory cytokines, microglia is a key modulator of neuroinflammation, neurodevelopment and CNS integrity (Bottcher et al., 2019). Microglia is also implicated in various neurological and psychiatric disorders, such as Alzheimer's disease (AD) (Das et al., 2019; Russo et al., 2019), schizophrenia (Sellgren et al., 2019; Wang et al., 2019) and depression (Lee et al., 2019). In addition to resting/inert M0 microglia, activated microglia have two major functional states – the pro-inflammatory M1 and anti-inflammatory M2 phenotypes (Huang et al., 2019).

As shown in Table 2, M1-microglia secrete cytotoxic factors (e.g., reactive oxygen species, ROS and nitric oxide, NO) and pro-inflammatory cytokines (e.g., IL-1 β , IL-6 and TNF- α) that inhibit neuronal growth, disrupt synaptic development and plasticity and, hence, contribute to the onset and pathogenesis of cognitive and mood disorders (Ramon-Duaso et al., 2019). In contrast, M2-microglia produce anti-inflammatory molecules (e.g., IL-4 and IL-10) that effectively attenuate deleterious effects of M1-microglia (Huang et al., 2019) (Table 2). However, despite the rapid progress in neuroglia research (Mayorquin et al., 2018; Numis et al., 2019; Verkhatsky et al., 2019b), their exact roles in humans and various animal species remain poorly understood, necessitating further in-depth studies and a wider spectrum of model organisms that may benefit the field by providing a critical, evolutionarily relevant context.

2. The utility of zebrafish models in neuroscience research

A small freshwater teleost fish, the zebrafish (*Danio rerio*) is rapidly emerging as a promising model organism in biomedicine (Genge et al., 2016; Meshalkina et al., 2017; Vasta et al., 2004) and human disease modeling (Cofer and Matthews, 2014; Gong et al., 2011; Mitsuhashi, 2018; Tian et al., 2019), including both experimental neurology (Kalueff et al., 2014; Stewart et al., 2015; Vaz et al., 2019) and biological psychiatry (Stewart et al., 2014). After mice, zebrafish are presently the second most used animal organism in biomedicine (Aleström and Winther-Larsen, 2016; Geisler et al., 2017; Hudson-Shore, 2016). Complementing mammalian models, multiple advantages of zebrafish models include the simplicity of genetic manipulations and gene editing (Gerlai, 2012; Le Bras, 2019), fully sequenced genome with high homology to human genome (Howe et al., 2013), rapid development (Grunwald and Eisen, 2002), well-recognized behavioral patterns (Kalueff et al., 2013) and a remarkable potential for high-throughput drug screening (Stewart et al., 2014).

Zebrafish models have been developed for multiple human brain pathologies, including neurodevelopmental, affective, psychotic and neurodegenerative disorders (Cosacak et al., 2017; Milanese et al., 2012). Contributing to our improved understanding of common, evolutionarily conserved mechanisms of CNS disorders, zebrafish are also widely used in behavioral research and CNS drug screening (Stewart et al., 2014), helping to establish functional links between neurochemistry and behavior in vertebrate species (Bugel and Tanguay, 2018).

Possessing superior neuro-regenerative ability than mammals, zebrafish and other teleost fishes have multiple neurogenic sites (e.g.,

spinal cord, optic tectum, retina, cerebellum and olfactory bulbs) vs. only hippocampal dental gyrus, lateral subventricular zone and olfactory bulbs in mammals (Ghosh and Hui, 2016; Trimpe and Byrd-Jacobs, 2016; Zupanc, 2011). Zebrafish neurogenesis involves RG cells with stem cell potential in adult stage, thus differing markedly from mammals, where RG is replaced by astrocytes during embryogenesis (Ghosh and Hui, 2016). Instead of astrocytes, zebrafish possess RG cells that are distributed in retina (Lenkowski and Raymond, 2014) and various parts of the CNS, including cerebellum (Kaslin et al., 2013), hypothalamus (Duncan et al., 2016), midbrain and spinal cord (Briona and Dorsky, 2014; Lindsey et al., 2019). Neuro-regenerative function of zebrafish RG involves the Wnt/ β -catenin signaling and growth factors, producing (like mammalian astrocytes) GFAP, TGF- β , FGF, GLT-1 and AQP4 (Alunni and Bally-Cuif, 2016; Lyons and Talbot, 2014). RG proliferation and differentiation are controlled by the several regulatory molecules, including aromatase B (AroB), brain lipid binding protein (BLBP) and proliferating cell nuclear protein (PCNA) (Diotel et al., 2016).

Zebrafish neurogenesis is important because of their high regenerative potential in brain areas, even including pallium – a homologue of mammalian neocortex (Alunni and Bally-Cuif, 2016). The neuro-regenerative properties of zebrafish depend on functional RG cells (Alunni and Bally-Cuif, 2016; Ghosh and Hui, 2016), the fish analogs of mammalian astrocytes. Zebrafish oligodendrocytes effectively repair injured axons by up-regulating myelin protein zero (mpz) (Bai et al., 2014). In general, microglia appears to be rather conservative across the vertebrate species, originating from the same source and commonly performing similar functions in the brain (Eyo and Dailey, 2013). Taken together, this raises a logical question of whether zebrafish models can advance neuroglia research by complementing, rather than merely mimicking, rodent and clinical evidence. In other words, can zebrafish be a useful, translationally relevant organism to study neuroglial pathobiology, yet offer novel translational insights based on zebrafish-specific features of their microglia biology? Here, we discuss neuroglia in humans, rodents and fish, and outline future directions of translational research into the role of neuroglia in CNS disorders using zebrafish models.

3. The effects of stress on neurons and glia

Stress is a common cause of CNS disorders, and glial cells are essential participants of brain stress responses (Aguirre et al., 2013; Clarke et al., 2019; Murphy-Royal et al., 2019; Pearson-Leary et al., 2016). In rodents, stress activates proinflammatory M1-microglia via toll-like receptors and inflammatory cytokines (e.g., IL-1 β) (Niraula et al., 2017), and causes astrogliosis with multiple deficient astrocytes and oligodendrocytes (Abbink et al., 2019). Microglia-mediated inflammation is common under chronic stress, lowering BDNF and elevating proinflammatory cytokines TNF- α , IL-6, IL-1 β , IL-12 and IL-18 (Bisht et al., 2018; Frank et al., 2019). Furthermore, changes in microglial cells number and their control of neurodevelopment are also crucial in rodent early developmental stress models (Johnson and Kaffman, 2018).

In contrast, the role of astrocytes in stress typically involves neuroprotection by controlling glutamate and ROS, mitochondrial recovery, metabolic stress, neuroimmune modulation and DNA homeostasis (Bylicky et al., 2018). For example, ROS are commonly neutralized by GFAP-positive astrocytes synthesizing antioxidants via the erythroid 2-related nuclear factor (Nrf2) pathway (Asanuma et al., 2019; Thorne et al., 2016). Likewise, stress-induced glutamate excitotoxicity in rat cortex is reduced by astrocytic glutamate decarboxylase 67 (GAD67) (Zhang et al., 2019), whereas astrocytes, activated by M2-microglia via the IL-10/TGF- β signaling pathway, induce an anti-inflammatory response and reduce pro-inflammatory cytokines IL-1 β , IL-6 and TNF- α (Norden et al., 2016).

Although much of our current knowledge of stress action on neuroglia is based on rodent and clinical evidence, zebrafish models may also

be useful (Sifuentes et al., 2016; Song et al., 2018). For example, in addition to multiple advantages discussed above, zebrafish have prominent behavioral stress responses, manifested as increased swimming speed, freezing (an immobility commonly observed at the bottom of the tank), frequent anxiety-like erratic movements, decreased aggression, and significantly increased 'protective' bottom swimming (Rehnberg and Smith, 1988; Kalueff et al., 2013). The main stress endocrine biomarker in humans, cortisol, is also used by zebrafish (Barcellos et al., 2007). However, stress hormones seem to be somewhat less involved in zebrafish neuroglia over-proliferation during stress, as this process is mostly associated with upregulation of fibroblast growth factor circuit in subpallium and cerebellum (Than-Trong and Bally-Cuif, 2015). On the one hand, there is a significant role of zebrafish RG in proinflammatory stress component via glia maturation factor beta (GMFB) overexpression in the telencephalon (Yin et al., 2018), similar to rodent findings (Hotta et al., 2005; Norden et al., 2016). On the other hand, the exact role of zebrafish glia in stress response and affective pathogenesis remains poorly understood, warranting further studies.

Recent evidence is only beginning to examine this problem. For example, in a novel zebrafish model potentially relevant to human post-traumatic stress disorder (PTSD), fish exposed to an acute severe 'combined' stressor display lasting anxiety-like behavior, elevated whole-body cortisol, as well as upregulated brain expression of genes encoding BDNF and its receptors (TrkB and P75), CD11b (a general microglial biomarker), COX-2 (an M1-microglial biomarker), CD206 (an M2-microglial biomarker), GFAP (a general astrocytal biomarker), C3 (an A1-astrocytal biomarker), S100 α 10 (an A2-astrocytal biomarker), as well as pro-inflammatory cytokines IL-6, IL-1 β , IFN- γ and TNF- α (Yang et al., 2020). As such, zebrafish not only successfully recapitulate lasting behavioral and endocrine symptoms of clinical stress disorders, but also implicate changes in neuroglia and neuroinflammation in long-term effects of stress pathogenesis (Yang et al., 2020).

In addition to stress per se, neuroinflammation and metabolic deficits contribute to the etiology of human affective disorders (Wang et al., 2020a, b). For example, exposing adult zebrafish to high-calorie diet (e. g., 2 % glucose + 10 % cholesterol) for 19 days induces type 2 diabetes, anxiety-like behavior, elevated whole-body cortisol and cytokines IFN- γ and IL-4, as well as upregulated expression of brain genes encoding CD11b, IL-6, TNF- α , GFAP, BDNF, p75 and TrkB (Wang et al., 2020a, b). Such findings reveal the overlapping nature of affective and metabolic pathogenesis and emphasize the role of neuroinflammation and neuroglia in the evoked affective states in zebrafish models (Wang et al., 2020a, b).

Finally, zebrafish affective states and their glial biomarkers are also sensitive to pharmacological modulation. For example, arecoline is a naturally occurring psychoactive alkaloid with agonism at nicotinic and muscarinic acetylcholine receptors, and its acute and chronic exposure is anxiolytic in zebrafish (Serikuly et al., 2021). Furthermore, chronic treatment of fish with 1 mg/L arecoline elevates brain expression of microglia-specific biomarker genes *egr2* and *ym1*, linking microglial mechanisms to long-term arecoline use (Serikuly et al., 2021). Likewise, kavalactones exert potent sedative, analgesic and anti-stress action in humans and rodents (Volgin et al., 2020), and their chronic administration exerts a sedative effect in zebrafish, also upregulating several microglial (*iNOS*, *Egr-2*, *CD11b*), astrocytal (*C3*, *C4B*, *S100a*), and pro-inflammatory (*IL-1 β* , *IL-6*, *TNF- α*) biomarker genes in the brain (Wang et al., 2020a, b). Collectively, these findings not only support translationally relevant, evolutionarily conserved behavioral and physiological responses to stress in zebrafish, but also provide novel important insights into potential role of neuroglial mechanisms in zebrafish affective states and their pharmacological modulation.

4. Zebrafish glia in CNS injury and regeneration models

Unlike mammals, zebrafish are uniquely capable of neuronal regeneration and functional recovery within 4–8 weeks after the spinal

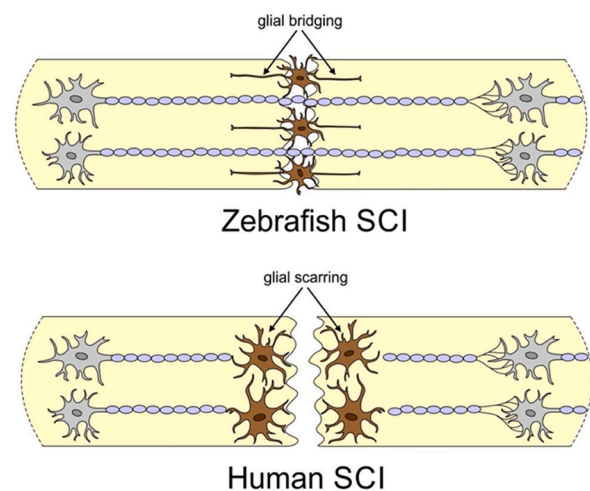


Fig. 1. The ability of neuronal recovery in zebrafish following the spinal cord injury (SCI, see text for details).

cord injury (SCI) (Becker et al., 2004; Dias and Göritz, 2018; Reimer et al., 2008), with both descending and ascending axons able to recover (Goldshmit et al., 2012b). The distinctive feature of zebrafish neuroregeneration is the ability of their glia to form 'bridges' that scaffold the axonal growth (Fig. 1) (Goldshmit et al., 2012b; Mokalled et al., 2016). In contrast, human glial scarring prevents spinal cord repair (Dias and Göritz, 2018; Wang et al., 2018), thereby giving poor prognoses for SCI patients (Burns et al., 2012). The population of glial cells in zebrafish spinal cord is represented by RG (Johnson et al., 2016), in adult fish homologous to mammalian astrocytes (Lyons and Talbot, 2014; O'Brown et al., 2018). Previous studies also compared zebrafish RG to mammalian astrocytes, abundant in the grey matter, dorsal and ventral horns of spinal cord, with thick projections between each other, structurally associated with neurons and blood vessels, and ensuring nutrition, protection (via BBB) and CNS signaling (Dermietzel et al., 2000; Kawai et al., 2001).

Notably, the population of RG within the zebrafish spinal cord is represented by two likely overlapping subgroups, the C-4 /GFAP-positive cells of brain ventricles (Tomizawa et al., 2000) and the A-22-positive cells of the ventral and dorsal horns (Kawai et al., 2001). There are also two major functional RG phenotypes in zebrafish: quiescent (qRG) and proliferating (pRG) (Lindsey et al., 2018). Like mammalian astrocytes, zebrafish RG are the main proliferating cells following the SCI (Goldshmit et al., 2012b). Furthermore, the RG bipolar morphology in zebrafish (vs. mammalian star-shaped astrocytes) explains axonal regeneration across the lesion, rather than glial scarring (Goldshmit et al., 2012b). Conserved across vertebrates, developmentally essential fibroblast growth factor (Fgf) (Böttcher and Niehrs, 2005; Katoh, 2016) and the connective tissue growth factor (Ctgf) (Hertel et al., 2000), both underlie the spinal cord regeneration in zebrafish via increased glial cells proliferation and bridge generation between two sides of the intersected spinal cord (Goldshmit et al., 2012b; Mokalled et al., 2016).

However, the exact role of oligodendrocytes and microglia in neuroregeneration in SCI models is poorly understood. For instance, mammalian neurotrauma models implicate oligodendrocyte-progenitor cells (OPC) in SCI, as they serve as the embryological source of mature oligodendrocytes what may potentially protect and recover injured axons (Chen et al., 2014; Franklin et al., 1996). Electrophysiological studies demonstrate that membrane properties of OPC in adult zebrafish spinal cord have many similarities (e.g., voltage-gated K⁺ and Na⁺ channels, as well as glutamate receptors) with those of mammals (Tsata et al., 2019). Importantly, these cells arrange the first responses to CNS injury in both zebrafish (Baumgart et al., 2012; Hui et al., 2010) and mice (Czeh et al., 2011).

Table 3

A brief summary of similarities and differences between zebrafish and rodent neuroglia.

Zebrafish	Rodents
Oligodendrocytes: similar Highly conserved across vertebrates (Kuhn et al., 2019; Preston and Macklin, 2015)	
Astroglia* Radial glia (RG) cells: Provide neuronal recovery without glial scarring (Verkhratsky et al., 2019) Interact with neurons in adult neurogenesis (Lyons and Talbot, 2014)	Astrocytes: Form glial scar due to neuronal injury (Verkhratsky et al., 2019) Indirectly influence adult neurogenesis via growth factors and other bioactive molecules (Cassé et al., 2018)
Induce neurodevelopment and arrange functional CNS geometry via the connections of their long processes with ECM proteins (laminins and integrins) (Long and Huttner, 2019)	Control neurodevelopment and organize CNS via matricellular proteins SPARC, hevin, TN-C, TSP and CCN3 (Jones and Bouvier, 2014) (Table 2).
Shared biomarkers (with rodent astrocytes) include GS, GFAP, TGF- β , FGF, GLT-1 and AQP4 (Alunni and Bally-Cuif, 2016; Anlauf and Derouiche, 2013; Lyons and Talbot, 2014)	Specific biomarkers include Aldh1L1, matricellular proteins SPARC, hevin, TN-C, TSP and CCN3 (Jones and Bouvier, 2014; Matias et al., 2019)
Specific biomarkers include AroB, BLBP and PCNA (Diotel et al., 2016; Schmidt et al., 2013)	
Microglia: similar Like oligodendrocytes, highly conserved across all vertebrates (Mazzolini et al., 2020)	

* Abbreviations: Aldh1L1 - aldehyde dehydrogenase 1 family, member 1, AQP4 - aquaporin 4, AroB - aromatase B, BLBP - brain lipid-binding protein, CCN3 - cellular communication network factor 3, ECM - extracellular matrix, FGF - fibroblast growth factor, GFAP - glial fibrillary acidic protein, GLT-1 - glutamate transporter-1, GS - glutamine synthetase, PCNA - proliferating cell nuclear antigen, SPARC - secreted protein acidic and rich in cysteine, TGF- β - transforming growth factor beta, TN-C - tenascin C, TSP - thrombospondin.

As a secondary responding element, larval zebrafish microglia are activated within 30 min after damage to a single motor neuron (Morsch et al., 2015). Although microglia are the main debris-clearing cells in the brain, they can travel outside through the spinal boundary, and vice versa (Green et al., 2019). This phenomenon may exacerbate pathological changes during SCI, since activated microglia have already been linked to various CNS deficits, including autism (Hanamsagar and Bilbo, 2017; Zeidán-Chuliá et al., 2014), AD (Hansen et al., 2018) and neuropathic pain (Zhuo et al., 2011). Finally, given multiple genetic and physiological similarities with mammals (Howe et al., 2013), identifying novel cells, genes and mechanisms underlying regeneration after zebrafish SCI may reveal novel approaches for translational studies. Understanding the role of each population of glial cells in such critical model system is also necessary for developing novel therapeutic approaches to redirect cellular reactions from glial scarring to bridge formation in patients with complete SCI.

5. Zebrafish glia in neurodegeneration, neurodevelopmental deficits and epilepsy

Neurodegenerative disorders, such as AD and Parkinson's disease, evoke severe brain damage and impaired neurogenesis in humans and mammals (Wirths, 2017). Because neurodegenerative processes are directly linked to neuroinflammation, brain microglia become an important player (Chitnis and Weiner, 2017). In mammals, the main proinflammatory cytokines produced by M1-microglia during neurodegeneration are TNF- α , IL-1 β and IL-6 (Prokop et al., 2013). In rodent AD-like neuroinflammation, the majority of M1-microglia biomarkers (Table 2) are activated by astrocytes (Chun et al., 2018). In contrast to humans and rodents, zebrafish renew their neurons during neurodegeneration (e.g., following A β _{1–42} peptide microinjections) (Saleem and Kannan, 2018). Such unique ability stems from close interactions between dying neurons and anti-inflammatory glial cells via the IL-4/STAT6 (signal transducer and activator of transcription 6) pathway that activates the proliferation of neural stem/progenitor cells (NSPCs) (Bhattarai et al., 2016; Yu et al., 2019). The NSPC plasticity in zebrafish AD models is also seemingly unique, since mammals do not respond this way, while fish involve the microglia/neurons-related NGFRA (receptor nerve growth factor receptor A)/NFkB (nuclear factor kappa B) pathway, triggering RG proliferation and differentiation, expressing both specific (e.g., PCNA) and non-specific (e.g., S100 β) biomarkers (Bhattarai et al., 2019, 2017).

Other important molecules expressed by neurons and microglia in vertebrate species are granulins (GRN) that control astrocytal and

microglial proliferation and functioning in both zebrafish and mice (Solchenberger et al., 2015). GRNs play a key role in zebrafish glial- and neurogenesis in the telencephalon, where the disruption of the GRN gene triggers neurodegeneration (Zambusi et al., 2020). In general, the NSC niche is more flexible in zebrafish than in mammals due to poor differentiation of RG, and may be controlled by various neuro-microglia signaling patterns, many of which are not yet fully understood.

Although mounting evidence implicates various CNS biomarkers in neurodevelopmental disorders (Zabegalov et al., 2019) and epilepsy (Kobylarek et al., 2019; Manford, 2017), relatively little is known about glial involvement in their pathogenesis, especially in preclinical models. For example, in young 'autistic' rats, glial cells (e.g., GFAP- and S100 β -positive astrocytes) exhibit mild neuroinflammation, whereas adults display fewer and more dysfunctional RG cells and immature neurons in the neurogenic brain areas, such as the hippocampus (Bronzuoli et al., 2018; Cope et al., 2016). In zebrafish, thalidomide disrupts neurodevelopment due to down-regulated cereblon (CRBN) expression, essential for both NSC and RG proliferation (Ando et al., 2019). In contrast, experimental epilepsy induces prominent neuroinflammation in zebrafish due to abnormal glutamate activity in astrocytes and the secretion of IL-1 β and TNF- α by the M1-microglia (Heuser et al., 2014). Epileptic zebrafish also show disturbed glutamate turnover by RG cells, indicating high extracellular glutamate, especially during seizures (Diaz Verdugo et al., 2019). Taken together, these findings directly implicate zebrafish neuroglia in some neurodevelopmental and neurological (e.g., epilepsy) disorders.

6. General discussion

Despite its growing utility in studying the role of neuroglia, the zebrafish has several limitations as a model organism. For example, due to genome duplication in teleost fishes (Bradford et al., 2017), data from zebrafish models of human diseases may sometimes be problematic to translate into human or rodent neurogenetic data (Davis et al., 2014). CNS analyses are complicated by unclear boundaries between closely located brain areas, such as the thalamus and basal ganglia (Vargas et al., 2011), and the small brain size in zebrafish may not afford enough morpho-anatomical distinction with high precision and granularity.

Importantly, both larval and adult zebrafish also express well-defined behaviors (Kalueff et al., 2013) in multiple neurobehavioral domains, including anxiety, locomotor activity, social behavior, aggression and cognition (de Abreu et al., 2020a, de Abreu et al., 2019; de Abreu et al., 2020b; Meshalkina et al., 2017) that are shared with mammals and humans.

Table 4

Selected open questions in zebrafish neuroglia neurobiology and its role in CNS disease modeling.

Questions
• How do differences in zebrafish and mammalian neuroglia impact preclinical central nervous system (CNS) studies? • Do individual behavioral differences translate into specific neuroglia profiles in zebrafish? • Does neuroglia differentiation foster or prevent modeling of human CNS disorders? • Do zebrafish neuroglial profiles differ in strain- and sex-specific manner? Do zebrafish display sex differences in neuroglia function, as rodents and humans do? • Are there overt differences between zebrafish and mammalian neuroglia responses following CNS drug exposure? • What is the predictive validity for pharmacological effects on neuroglia and its models of CNS disorders in zebrafish? • What is the role of neuroglia in epigenetic modulation in zebrafish CNS? • Is there any difference in neuroglial structure between wild and laboratory zebrafish? • Can current genetic manipulations (e.g., CRISPR*) with human or rodent glial cells assist the treatment of neurodegenerative disorders (e.g. Alzheimer's disease, AD)? • What are neuroglia's proteomic signatures underlying CNS function and dysfunction? • Are there any connections between neuroglia (in both fish and mammalian) and apoptosis mechanisms in neurodegenerative disorders? • How does zebrafish age (e.g., adult vs. aged) influence on neuroglia functionality and apoptosis? • Do genes and proteins controlling glial bridge formation exist in humans and rodents? • What is the role of microglial cells migration in neuronal pathways of the spinal cord injury recovery? • What is the impact of gut microbiota on neuroglia in zebrafish models of CNS disorders?

However, compared to well-established rodent behavioral models, zebrafish neurophenotyping batteries are relatively less complex and validated, primarily including anxiety tests (e.g., the novel tank test, open field, the light-dark box (Song et al., 2016)) and sociality assays (Buske and Gerlai, 2011; Stewart et al., 2015). For example, zebrafish locomotor activity can be evaluated in the open field or novel tank tests, analyzing swimming distance, velocity, mobility and angular motion characteristics (Kysil et al., 2017; Stewart et al., 2012). Likewise, fish anxiety-like behavior can be examined in the novelty-based paradigms, such as the novel tank test and light-dark box test, assessing fish exploration in 'aversive' top (vs. 'protective' bottom) or light (vs. dark) sections, respectively (Kysil et al., 2017; Stewart et al., 2011). Zebrafish social behaviors can be evaluated by assessing their cohesion in the shoaling test, or social preference in the social preference test (Pham et al., 2012). Finally, cognitive phenotypes in zebrafish can be evaluated using various aquatic T-, Y- or plus-mazes, conceptually similar to rodent memory and learning tasks (Meshalkina et al., 2017; Stewart and Kalueff, 2012).

In terms of neuroglia studies, the most critical limitation of zebrafish is structural-functional distinction between zebrafish macroglia (RG) and mammals macroglia (astrocytes; Table 3), which complicates using zebrafish in some neurological studies. Moreover, zebrafish neuroglia often participate in neurodegenerative processes by initiating anti-inflammation and neurogenesis (Bhattacharai et al., 2019, 2017) – i.e., in a manner seemingly opposite to that of mammals, thus, again complicating direct translation from fish tanks to clinical bedside.

In summary, glia is a critical factor in neuronal development and repair both in mammals and fish (Verkhatsky and Nedergaard, 2018), including bridging processes in fish CNS regeneration and glial scar formation in mammals (Verkhatsky et al., 2019a), as well as Fgf-induced axonal regeneration after spinal cord injury (Goldshmit et al., 2012a). Recent data suggest a significant role of premature NSCs, such as RG, in adult neurogenesis in vertebrates (Ghosh and Hui, 2016; Iismaa et al., 2018). In zebrafish, adult neurogenesis is very robust due to RG abundance in the CNS (Zupanc, 2011). Thus, although NSCs share multiple cellular and molecular traits with mammalian adult NSCs, their distribution in the zebrafish adult CNS is much wider than in mammals (Ghosh and Hui, 2016). Compared to mammalian NSCs, in adult zebrafish they are more effectively activated and involved in neuro-regeneration (Alunni and Bally-Cuif, 2016), especially overt in the SCI models (Reimer et al., 2008). Thus, studying the role of zebrafish neuroglia in adult neurogenesis may expand our understanding of neuroprotection and neuroregeneration (Duncan et al., 2016), in turn providing important insights into neuroglia-mediated mechanisms of CNS disorders.

Brain activity and connectivity are altered drastically in neuropathies (e.g., epilepsy or stress) (Cauda et al., 2018), and preclinical rodent studies have already linked neuroglia to various CNS disorders (Blume

et al., 2019; Bogie et al., 2014; Hotta et al., 2005; Ritzel et al., 2015; Zhang et al., 2019). A powerful novel vertebrate system for modeling neurological and psychiatric disorders (Copmans et al., 2017; de Abreu et al., 2020b, de Abreu et al., 2019; Meshalkina et al., 2018), zebrafish show partially shared, and partially distinct aspects of neuroglia neurobiology from those of mammals (Alunni and Bally-Cuif, 2016), thereby becoming a valuable tool in elucidating the role of glia-glia and glia-neuronal interactions in CNS disorders (Verdugo et al., 2019). For example, zebrafish stress studies reveal the evolutionarily conserved neuroinflammatory neuroglia patterns, involving proinflammatory RG and M1-microglia (Yin et al., 2018), like in rodents (Niraula et al., 2017).

Interestingly, zebrafish do not display overt neuroinflammation and neurodegeneration in AD models, where the anti-inflammatory M2-microglia rapidly activates NSPC proliferation and differentiation (Bhattacharai et al., 2016), unlike mammals (Prokop et al., 2013). However, neurodevelopmental (e.g., autism) and neurological (e.g., epilepsy) disorders can affect zebrafish and rodent neuroglia in the same way, since the RG cell proliferation decreases in autism (Ando et al., 2019; Cope et al., 2016), and epilepsy is associated with neuroinflammation and glutamate toxicity in both models (Diaz Verdugo et al., 2019; Heuser et al., 2014).

Furthermore, as the recently developed methods of real-time neuroglia analyses employ advanced high-resolution imaging, cell sorting, optogenetics and novel pharmaceuticals, applied to preclinical studies (Butt and Verkhatsky, 2018), zebrafish present remarkable advantages (over mammals) for high-resolution in vivo imaging and genetic analyses, accompanied by studies related to glial development and function (Lyons and Talbot, 2014). For instance, these powerful methods enable tracing the patterns of myelination in zebrafish by in vivo screening of larval oligodendrocyte development and biology (Early et al., 2018). Likewise, powerful genetic techniques probe the development of neurogenic areas of adult zebrafish brain, highlighting, for example, the specific neuroepithelial progenitor cell line in the embryonic neural tube (Galant et al., 2016).

In addition to multiple genetic factors, epigenetic control plays an important role in neuroglia biology, as shown in studies of age-related retinopathies and zebrafish Müller glia (Corso-Díaz et al., 2018). Moreover, various pharmaceutical agents are often used as epigenetic modulators of zebrafish neuroglia (Lyons and Talbot, 2014). For example, exposing zebrafish to cadmium increases cell death and neuronal apoptosis, down-regulates the expression of GFAP and reduces GFAP-positive glial cells in the brain (Monaco et al., 2016). In contrast, estrogens evoke constitutive neurogenesis and regeneration of neuroglia in zebrafish (Pellegrini et al., 2016), thus showing how various chemical agents can potentially and bidirectionally modulate zebrafish neuroglia.

Overall, the evidence discussed here suggests zebrafish as a promising experimental model organism to study the role of neuroglia in CNS

pathogenesis and disease modeling. However, as many questions remain open in this field (Table 4), future studies are necessary to further address the involvement of zebrafish neuroglia in complex CNS processes and disorders.

Declaration of Competing Interest

The authors have no conflicts of interest to disclose.

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