Title: Comparative Functions of the Endogenous Neural Stem Cell Secretome Across Species through Neurodevelopment and Disease

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1. Introduction

Neural stem cells (NSCs) have the potential to transform treatment of illness and injury in the central nervous system (CNS). NSC-based therapies have been investigated as treatments for numerous diseases and injuries, including stroke, multiple sclerosis, Alzheimer's disease, traumatic brain injury, spinal cord injury and Parkinson's disease (Willis et al., 2020b). While the generation of new neurons, astrocytes or oligodendrocytes is frequently the goal of such pre-clinical and clinical work, NSC-derived secreted products (i.e. their secretome) can also aid in a variety of cellular processes, including host cell survival, neuroplasticity and neuroimmune modulation (Zhang et al., 2020). This concept of the paracrine function of stem cells is well accepted as a major mechanism by which stem cells from the mesenchymal lineage interact with neural tissue (Badyra et al., 2020). However, the contents and potential of the NSC secretome are still emerging topics of research.

Unlike mesenchymal and most other non-neural stem cells, NSCs reside in the healthy mammalian CNS throughout development and aging (Kozareva et al., 2019). Natural NSC niches provide an endogenous source of NSCs to support healthy tissue homeostasis and a potential reservoir for supporting CNS recovery from damage (Marsh and Blurton-Jones, 2017). Endogenous NSCs are widely studied for their role in CNS development and the production of adult-born neurons throughout life in many species (Snyder, 2019). They also produce a diverse secretome composed of growth factors, cytokines, chemokines, morphogens, microRNAs (miRNAs), and other products (Shoemaker and Kornblum, 2016). Some secretome products, particularly soluble proteins, are secreted via exocytosis of secretory vesicles. Others, such as miRNAs, are packaged into small, lipid-bound extracellular vesicles (EVs) (e.g. exosomes), before release in to the extracellular space (Vogel et al., 2018). Studies of these endogenous NSC secreted factors provide a window in to how NSCs can interact with CNS tissue, thereby deepening fundamental understanding of the function of NSCs and how they might be used therapeutically.

This review will focus on research to date investigating the functions of the endogenous NSC secretome across neurodevelopment in health and disease. We will focus primarily on rodent models, where most data are available. However, we also add cross-species comparisons with the limited findings in humans and zebrafish. Across the lifespan, we categorize the potential functions of the NSC secretome into 3 categories: autocrine, paracrine and bidirectional. Autocrine signaling is defined as secreted signaling from one NSC to another, or one NSC to itself. Paracrine signaling is defined as secreted signaling from NSCs to neighboring cells, which can include cells derived from other lineages (e.g. endothelia, microglia), as well as NSC progeny such as progenitor cells or immature neurons. Bidirectional functions include the more complex interchange between NSCs and other cells in which an NSC secretome product alters a neighboring cell in ways that feedback to change NSC function. We find evidence of all three categories of secretome function in endogenous NSCs. However, we also find notable gaps, particularly in terms of the paracrine and bidirectional functions of adult niche NSCs. Studies of endogenous NSC paracrine functions in disease are also particularly rare. In sum, we find that while ample evidence indicates a potent role for the endogenous NSC secretome in regulating neural tissue, numerous open questions remain as challenges for future research.

2. Neural Stem Cell Secretome Across Neurodevelopment: evidence from rodents

2.1 NSC secretome in the pre and peri-natal CNS

Pre and peri-natal development of the CNS is a highly conserved process among vertebrates (Semple et al., 2013). In rodents such as mice, CNS generation begins at embryonic day (ED) 9 with the formation of the neural plate and then neural tube from ectodermal tissue (Rice and Barone, 2000). Following the involution and fusion of the neural tube, neuroepithelia cells in the newly formed ventricular zone divide to create an expanding pool of radial glia neural stem cells (rgNSCs). rgNSCs extend bipolar processes to contact both the ventricular space and the surface of the developing cortical plate, the latter of which eventually serves as a migration scaffold for progenitors (Noctor et al., 2004). Ventricular rgNSCs in the nascent mammalian CNS initially favor symmetric divisions, thereby expanding the rgNSC pool (Haubensak et al., 2004). Over the course of the remaining embryonic days, they shift to favor asymmetric divisions that produce unipotent neuronal, astroglial and then

oligodendoglial progenitor cells, in that temporal order (Qian et al., 2000). As a result of this sequential production of fate-restricted progenitors, most neurogenesis in the cortical and subcortical areas is complete by birth while astrocyte and oligodendroglial generation occurs mostly in the first postnatal month (Miller and Gauthier, 2007).

2.1.1 Pre- and peri-natal NSC secretome autocrine regulation.

The rodent rgNSC secretome is well established to regulate rgNSC self-renewal and production of progenitors in an autocrine manner, thereby supporting healthy CNS development. Perhaps one of the best-known examples of such rgNSC autocrine self-regulation is rgNSC self-stimulation via secreted sonic hedgehog (SHH). SHH is a morphogen that is produced and secreted by a variety of tissue and cell types, including rgNSCs in the developing brain. Mutations in *shh* can impact multiple tissues that rely on this molecule, both within and outside the CNS. SHH production by rgNSCs varies by anatomical region and developmental time, resulting in a tightly controlled reliance on self-generated SHH for proliferation and differentiation that is regionally-specific (Komada et al., 2008). For example, rgNSC-specific SHH knockdown decreases asymmetric production of progenitors from rgNSCs in the dorsal telencephalon and neocortex, but not in the ventral telencephalon (Komada et al., 2008; Shikata et al., 2011). Regional differences in SHH expression also influence neuronal differentiation and neuronal positioning as rgNSC-specific SHH knockdown increased neuronal differentiation in the neocortex but had no impact on formation of the ventral telencephalon (Komada et al., 2008). This case of SHH autocrine signaling illustrates a critical consideration in investigations of the NSC secretome. SHH, like many other secreted proteins, is made by multiple cell types and in multiple anatomical locations. Yet, as the regionally-specific autocrine role of SHH in rgNSCs shows, the functional role of a secreted protein for a target cell can hinge on which cell makes that protein. Factors such as diffusion/transport distance of secreted proteins and varying receptor expression levels likely play roles in determining the relative effects of the same protein made by different cells, though this is an area in need of further investigation. Still, the case of spatially-specific SHH autocrine signaling highlights the importance of evaluating secretome function cell-specifically when attempting to understand its functional impact.

Several other factors have been implicated in autocrine rgNSC self-regulation, including platelet derived growth factor (PDGF), chondroitin sulfate proteoglycans (CSPGs), apolipoprotein E (ApoE) and pituitary adenylate cyclase-activating peptide (PACAP). PDGF is a pleiotropic growth factor expressed by cells from both the mesodermal and ectodermal lineages (Ding et al., 2013; Funa and Sasahara, 2014). While it was originally discovered to influence blood vessel formation and maturation, PDGF is also critical for neural development (Fruttiger et al., 2000; Hellström et al., 1999). In the developing CNS, PDGF is expressed by both neurons and rgNSCs where it is reported to act as a mitogen for immature neurons (Erlandsson et al., 2006; Yeh et al., 1991). Experimental evidence from cultured E15 rat rgNSCs, in PDGF free media, indicate that self-expressed PDGF may also regulate the rgNSC pool in an autocrine manner (Erlandsson et al., 2006). Inhibition of PDGF receptors with a tyrosine kinase inhibitor suppressed cultured rgNSC proliferation and significantly reduced differentiation into neurons and oligodendrocytes, but not astrocytes (Erlandsson et al., 2006). This example of autocrine PDGF signaling in rgNSCs in vitro highlights the importance of cell culture experiments for investigating the functions of the NSC secretome. PDGF signal, and many other NSC-secreted factors, influence multiple, interacting facets of CNS development. Cell culture experiments provide a readily tractable experimental system that allows for investigation of rgNSCs in isolation from other cell types. While in vivo, cell-specific experiments remain critical as well, in vitro experiments provide a unique window into autocrine signaling that has fueled progress in this field substantially.

rgNSCs also express and secrete a family of proteoglycans known as CSPGs, which modulate many processes related to CNS development, such as cell division, adhesion and migration (Holst et al., 2006; Kubota et al., 1999; Mizuguchi et al., 2003). While CSPGs are expressed throughout the body and have been studied for their role in cartilage structure and function and tumor growth, the main focus of work on CSPGs has been on their role in neurodevelopment (Silver and Silver, 2014; Watanabe et al., 1998). RgNSCs and their progenitors express and secrete multiple types of CSPGs,

including aggrecan, neurocan and phosphocan, and rgNSC-secreted CSPGs signal within the rgNSC pool to enhance survival (Holst et al., 2006; Kabos et al., 2004; Tham et al., 2010). Given the production of CSPGs by multiple cell types and their effects on multiple cell types in the developing CNS, cell culture experiments have been critical to understanding the autocrine role of CSPGs in rgNSCs. Tham et al. first established that CSPGs are detectable by mass spectrometry in the conditioned media of mixed rgNSC and progenitor neurospheres derived from the embryonic mouse CNS, indicating the potential for autocrine signaling. Subsequent experiments showed that supplementing neurospheres with CSPGs increased rgNSC survival and proliferation while CSPG inhibition led to exhaustion of the NSC pool (Tham et al., 2010). Whether autocrine CSPG support is mediated by increasing quiescence or cell survival remains to be established. Future work is also necessary to determine the distinct roles of each type of CSPG in regulating rgNSCs. However, these findings suggest that growth factors and morphogens are not the only factors within the rgNSC secretome that play a role in autocrine signaling.

Similar to CSPGs, autocrine ApoE plays a significant role in stimulating rgNSC survival. ApoE is a lipid transporter expressed most notably in the liver, where it regulates lipid metabolism (Marais, 2019). In the adult CNS, ApoE is most highly expressed and secreted by astrocytes, and it has been established as a negative regulator of cell proliferation (Nathan et al., 1994; Pitas et al., 1987). During development, though, ApoE also has a temporally-defined expression pattern in rgNSCs, where expression is lower throughout rgNSC pool expansion and neurogenesis but raises during the shift towards gliogenesis (Gan et al., 2011). In cultured mouse forebrain rgNSCs, self-secreted ApoE stimulated neurosphere formation by facilitating NSC self-renewal, possibly through MAPK/Erk signaling (Gan et al., 2011). In addition, rgNSC specific ApoE knockdown *in vivo* increased activation of the rgNSC pool in the developing DG of mice (Tensaouti et al., 2018), demonstrating that the functional role of rgNSC-produced ApoE persists in the context of the full in vivo niche.

In addition to factors that stimulate rgNSC survival, proliferation and differentiation into neural progenitors, rgNSPCs also secrete factors that promote gliogenesis. PACAP is a pleiotropic neuropeptide expressed throughout the CNS and periphery during development (Tatsuno et al., 1994; Zhou et al., 2002). Cortical rgNSCs derived from embryonic mice were found to express both PACAP and its receptor PAC1 in vitro (Nishimoto et al., 2007). PAC1 stimulation by PACAP led to rgNSC proliferation and differentiation into the astrocytic lineage, suggesting that autocrine PACAP-mediated signaling may regulate the switch from neurogenesis to gliogenesis in the developing CNS (Nishimoto et al., 2007). Yet, studies of PACAP and its role in autocrine signaling in the rgNSC pool have yet to be fully resolved. An early study found that PACAP stimulation inhibited proliferation and promoted neurogenesis in cultured rat neuronal progenitors (Lu and DiCicco-Bloom, 1997). When administered into the embryonic CNS, PACAP similarly inhibited proliferation at ED13.5 (Ohtsuka et al., 2008). However, when given at ED15.5 in rats, it did not promote neurogenesis as it did in vitro; rather it reduced cortical neurogenesis (Suh et al., 2001). Together, these findings suggest a possible temporal regulation of PACAP in its effects on the rgNSC pool and CNS development. Differential effects of PACAP signaling may be attributed to expression of the three PACAP receptors, PAC1, VPAC1, and VPAC2, that mediate PACAP function in the CNS (Christophe, 1993; Muller et al., 1995; Tatsuno et al., 1994; Zhou et al., 2002). Previous studies have identified PAC1 as the main receptor expressed by rgNSCs in development, but VPAC1 and VPAC2 have not been as thoroughly studied in the developing brain and may influence how rgNSC autocrine PACAP signaling impacts neurodevelopment. The temporal differences in PACAP signaling and how it impacts the rgNSC pool highlights the importance of evaluating potential ligand/receptor signaling pathways when determining the functional significance of the NSC secretome.

2.1.2 Pre- and peri-natal NSC secretome paracrine regulation.

The paracrine role of the rgNSC secretome is generally less extensively studied than its autocrine role. Nonetheless, examples of well understood paracrine functional roles of the rgNSC secretome exist, the most prominent of which is induction of CNS vascularization. Vascularization of the CNS is critical for development, yet because endothelia cannot be produced from neuroectodermal tissue (James and Mukouyama, 2011), they must be attracted from the mesoderm to vascularize the

CNS. The rgNSC secretome drives vascularization of the CNS through the expression and secretion of vascular endothelial growth factor (VEGF), a powerful chemoattractant and mitogen for endothelia (Gerhardt et al., 2003; Haigh et al., 2003; Raab et al., 2004). Specifically, VEGF from rgNSCs and their progenitors signal to VEGFR2 on endothelial tip cells to induce tip cell migration and to VEGFR2 on stalk cells to stimulate their proliferation (Di Marco et al., 2020; Gerhardt et al., 2003; Komabayashi-Suzuki et al., 2019; Miyama et al., 1997; Ruhrberg et al., 2002; Shen et al., 2004). The end result is coordinated growth and migration of endothelia-lined vessels towards zones of rgNSC and progenitor production. The specific role of rgNSC- and progenitor-produced VEGF in eliciting vascularization is essential. Even hemizygous loss of *vegf-a* in rgNSCs leads to embryonic lethality due to a disruption of CNS vasculature development (Ferrara et al., 1996). Vascular chemoattraction towards rgNSC-expressed VEGF also drives NSC activation in the developing brain as angiogenic sprouting and the ingression of tip cells induces asymmetric rgNSC division and the production of neurons that form the cortical layers (Karakatsani et al., 2019). These findings establish that rgNSC-secreted factors can not only influence migration of non-neural tissue into the developing CNS, but also influence their own regulation through bidirectional cellular communication.

The rgNSC secretome may also play a paracrine role in CNS immune system development and regulation, though this is generally less well-understood than its role in vascular development. Microglia are the resident neuroimmune cells of the CNS but, similar to endothelia, the neuroectoderm does not have the potential to produce microglia. Microglia are instead derived from the yolk-sac and then migrate to the CNS during gestation (Thion et al., 2018). A role for the rgNSC secretome in microglia migration and regulation within the developing CNS has been suggested by recent findings (Morton et al., 2018). Using next generation small RNA sequencing of EVs derived from conditioned media of cultured P0 SVZ rgNSCs, Morton and colleagues (Morton et al., 2018) identified high expression of several miRNAs known to regulate microglia morphology and physiology, including miRNAs let-7, miR-9 and miR-181 (Kumar et al., 2015; Lehmann et al., 2012; Zhang et al., 2015). They further observed that ingressing microglial cells took up fluorescently-tagged EVs derived from cultured NSCs, and that EV incorporation was correlated with a shift from a stellate to a more rounded morphology by microglia. Consistent with these morphological changes, cultured primary microglia treated with rgNSC EVs also showed increased expression of IL-1α, IL-1ß, and IL-6, indicating greater proinflammatory activity. Interestingly, the conditioned medium from EV-treated microglia significantly inhibited proliferation of cultured SVZ-derived rgNSCs, once again indicating that the NSC secretome may indirectly self-regulate by driving bi-directional communication with other niche cell types. One notable remaining challenge is to show a causal link between NSC-EVs synthesized *in vivo* and microglial regulation. The majority of the experimental work above relied on production and concentration of EVs *in vitro*, which may or may not reflect physiological levels of NSC-EV production. This is a common obstacle in studies where cell-specific manipulation is technically challenging, such as is the case for production of EVs, and remains an area for future investigation and technique development.

2.1.3 Pre- and peri-natal NSC secretome: other factors and open questions.

There are likely many other rgNSC secretome factors that remain to be discovered. A broad, unbiased identification of the rgNSC secretome is currently lacking *in vivo*, but *in vitro* approaches provide some clues to the possible diversity of rgNSC secreted proteins. For example, (Severino et al., 2013) performed liquid chromatography tandem mass spectrometry on the conditioned media of cultured NSCs from the immortalized NSC line mes-c-myc A1, which were derived from the mesencephalon of ED 11 mice as they underwent differentiation into neurons. This study identified 104 non-redundant proteins and found that a significant percentage of the proteins were related to nervous system development and function, cell adhesion and cell survival. The same group then used a magnetic bead-based multiplexed immunoassay to further investigate the secretion of cytokines and chemokines by proliferating rgNSCs (Colucci-D'Amato et al., 2015). They found IFN-γ, IL-1α, IL-6, IL-12, IL-13, IL-5, and IL-10 were released by rgNSCs. Of these factors, IL-6 has been proposed to be a regulator rgNSC quiescence but exhibits pleitopic effects depending on the cell (Hirano et al., 1990; Mathieu et al., 2010). The functional roles of rest of these factors in autocrine regulation of rgNSCs and

paracrine regulation of the developing nervous system remain to be investigated. While the methodologies used to identify the secretome in these studies are biased towards only the most highly expressed factors within the secretome, these studies identify several factors and potential cellular process that the rgNSC secretome is poised to regulate in the developing rodent brain.

In summary, the rgNSC secretome in the developing CNS plays a pivotal role in NSC regulation, endothelial migration and possibly neuroimmune regulation (Figure 1). RgNSC autocrine factors are often spatially and temporally specific in their regulation of the rgNSC pool. RgNSCs also produce paracrine signaling factors that may function to attract and regulate other cell types within the developing CNS. This paracrine signaling between niche cells can be bi-directional, resulting in indirect regulation of the rgNSC pool. Though it is clear that the rgNSC secretome is a major component of healthy CNS development, this field has many questions left unexplored. Future studies of the spatial and temporal changes in the rgNSC secretome (both what comprises the secretome and in what quantities factors are produced) are needed to better elucidate how the rgNSC secretome influences healthy neurodevelopment. Further development and refinement of methods for spatially and temporally specific manipulation of the rgNSC secretome will also facilitate better understanding of the role of rgNSC secreted factors in neural development.

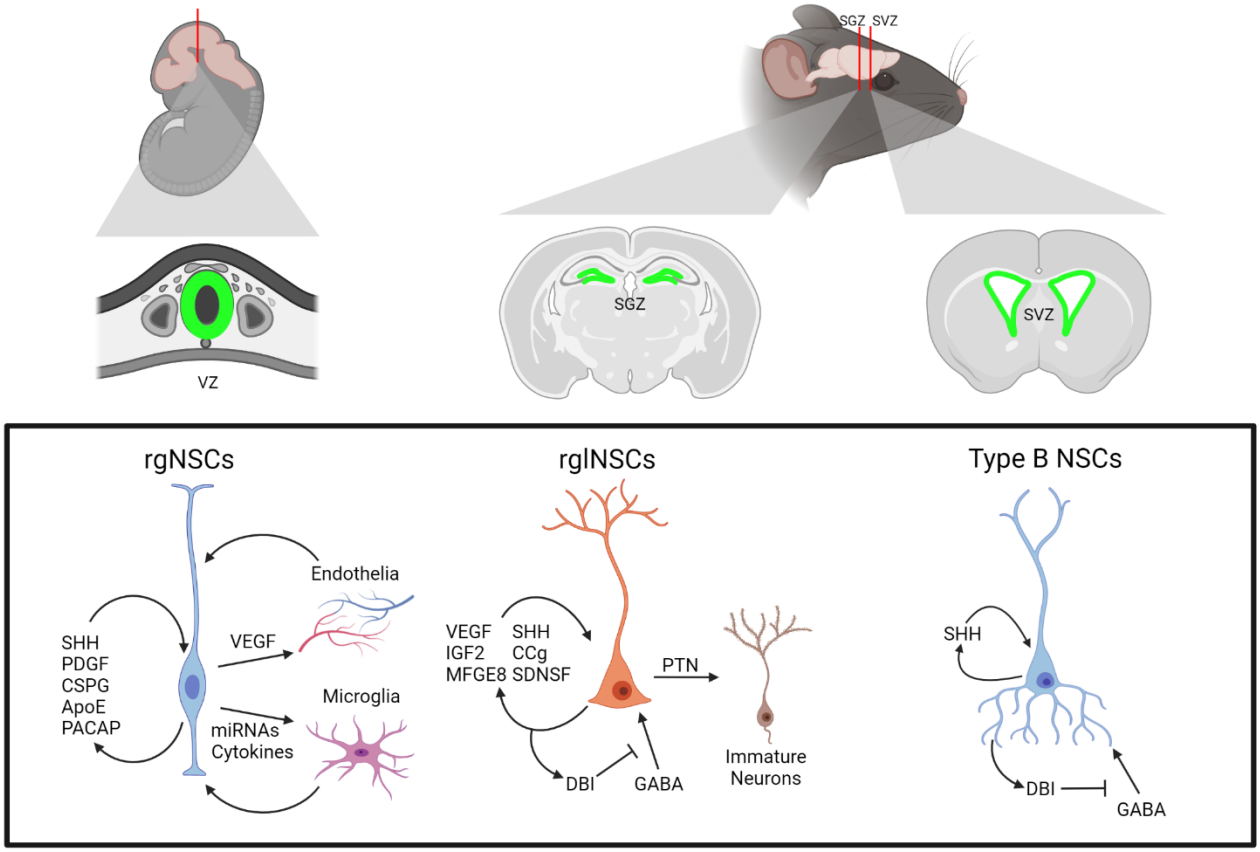

Figure 1: Autocrine and paracrine signaling of rodent NSC secretomes. Summary of factors and their autocrine and paracrine functions that have been identified in secretomes of rgNSCs in the developing CNS, rglNSCs in the adult SGZ, and type B NSCs in the adult SVZ in rodent models. VZ, ventricular zone; SGZ, subgranular zone; SVZ, subventricular zone, rgNSC, radial glia neural stem cell; rglNSC, radial glia like neural stem cell; type B NSC, type B neural stem cell; SHH, sonic hedgehog; PDGF, platelet derived growth factor; CSPG, chondroitin sulfate proteoglycans; ApoE, apolipoprotein E; PACAP, pituitary adenylate cyclase-activating peptide; VEGF, vascular endothelial growth factor; miRNA, micro RNA; IGF2, insulin like growth factor 2; MFGE8, Milk fat globule-EGF factor 8; CCg, glycosylated Cystatin C; SDNSF, stem cell-derived neural stem/progenitor cell supporting factor; DBI, diazepam binding inhibitor; GABA, gamma-aminobutyric acid PTN, pleiotrophin. Created with BioRender.com

2.2 NSC secretome in the juvenile CNS.

The rodent CNS continues to develop following birth and throughout adolescence. Postpartum neurodevelopment in rodents is marked by a decline in neurogenesis (Mathews et al., 2010) and progressive confinement of NSCs into neurogenic niches, the most prominent of which are the DG of the hippocampus and the SVZ lining the lateral ventricles (Bond et al., 2015) (Figure 2). The formation

of these two niches is qualitatively different and the NSCs that establish them are derived via different ontological processes.

The adult rodent DG neurogenic niche relies on an NSC class known as radial glia-like NSCs (rglNSCs), which reside in the subgranular zone of the DG throughout life. The DG rglNSC pool matures from a subset of Hopx+ precursors that originate in the neuroepithelium. These select precursors detach from the ventricle around ED15.5 and migrate to the nascent hippocampus via the dentate migratory stream over the first several postnatal weeks (Berg et al., 2019). While the hippocampal formation is established by ED 17, granule cells that form the DG continue to be produced in large quantities throughout early adolescence until ~postnatal day (PD) 21 (Urbán and Guillemot, 2014). RglNSCs largely complete migration to the subgranular zone of the DG by PD14 (Nicola et al., 2015), where they continue to mature into a more adult phenotype (Hochgerner et al., 2018). Around the end of the first postnatal month, a resolved SGZ is evident with largely quiescent rglNSCs and proliferating intermediate progenitor cells (IPCs) that continue to generate neuroblasts that mature into new granule neurons, though at a greatly diminished rate compared to the early peri-natal period (Gilley et al., 2011; Hochgerner et al., 2018). Through aging, rglNSC quiescence deepens and IPC proliferative capacity diminishes even further (Harris et al., 2021).

The adult SVZ niche relies on an NSC class known as type B NSCs. SVZ type B NSCs are derived from a subpopulation of rgNSCs set aside during embryonic development. RgNSCs enter a quiescent state between ED13 and ED15 and mature into early type B NSCs between ED17.5 and PD21 (Fuentealba et al., 2015; Furutachi et al., 2015). During early postnatal development, the maturing type B NSCs are responsible for the production and supply of both astrocytes and oligodendrocytes for most of the developing brain (Levison and Goldman, 1993). They also give rise to neurons that form the medial and lateral septum and striatum depending on their spatial location before entering their adult quiescent state (Obernier and Alvarez-Buylla, 2019). In adulthood, type B cells settle into an architecturally defined niche, where they extend processes to contact both the CSF in the lateral ventricle and local blood vessels (Fuentealba et al., 2012). Though they are more quiescent than early post-natal, maturing B cells, they still reactivate to give rise to type C IPCs, which in turn divide to create neuroblasts (type A cells). Those type A neuroblasts migrate away from the SVZ via the rostral migratory stream to eventually mature into interneurons in the olfactory bulb. Interestingly, SVZ type B NSCs maintain a spatial organization through transcription factor expression, leading to heterogeneity in the type of interneuron produced by NSCs in the adult SVZ that varies by anatomical location (Obernier and Alvarez-Buylla, 2019).

During this early postnatal development time window, when the neurogenic niches are forming, there is little known about the NSC secretome of either rglNSCs or type B NSCs. Previous reports have identified transcriptional changes that occur with maturation of NSCs in both the SVZ and SGZ (Hochgerner et al., 2018; Yuzwa et al., 2017). Yet, to the best of our knowledge, there has been no attempt to characterize the secretome of maturing NSCs as they to adopt new, tissue specific identities that are unique to their neurogenic niches. The field of maturing NSC secretome function therefore remains an almost completely open target for future research.

2.3 NSC secretome in the adult CNS

2.3.1 Adult NSC secretome autocrine regulation.

A vast literature supports the importance of niche-derived factors in regulating each aspect of the neurogenic process in both the adult SVZ and the adult DG (Niklison-Chirou et al., 2020; Urbán et al., 2019; Vicidomini et al., 2020). Relatively few studies, by comparison, probe the role of the adult NSC secretome in regulating themselves or their niche in return. Among existing studies, autocrine signaling has thus far emerged as the most well-defined role of the adult NSC secretome in both major adult niches. Known autocrine factors that play a role in self-sustaining NSCs in the adult brain include VEGF, IGF2, MFGE8, SHH, Wingless and Int-1 (WNT), glycosylated cystatin C (CCg) and stem cell-derived neural stem/progenitor cell supporting factor (SDNSF). While some of these autocrine factors are conserved in both niches, such as SHH, the expression and secretion of others in the secretome, like WNT, VEGF, MFGE8 etc., appear to be niche-dependent. These differences may be due to

differences in soluble factors released by other cells within each niche or to divergence in adult DG and SVZ NSC pool formation/maturation.

VEGF is a pleiotropic, soluble factor that is expressed by adult DG rglNSCs, as well as other cell types. Despite VEGF production by other CNS cell types, such as astrocytes, self-produced VEGF is necessary to maintain the balance between quiescence and activation among rglNSCs (Kirby et al., 2015). In our previous work, we established this autocrine role of rglNSC-VEGF by showing that cell-specific loss of VEGF in adult NSCs and their progenitors led to a surge in rglNSC proliferation followed by exhaustion of the rglNSC pool (Kirby et al., 2015). This phenotype of disrupted quiescence and exhaustion occurred both in cultured DG rglNSCs and in the adult mouse hippocampus, suggesting it was not reliant on bidirectional signaling with neighboring cell types. VEGF expression was not found to be a prominent feature of SVZ NSCs, suggesting this secretome-mediated self-maintenance mechanism is unique to hippocampal rglNSCs.

IGF2 is a soluble protein secreted most prominently by the liver (Adamek and Kasprzak, 2018). IGF2 is also synthesized locally in the hippocampus, where it is necessary to maintain the rglNSC pool and support the survival of adult-born neurons (Bracko et al., 2012; Ziegler et al., 2012). At least some portion of this local IGF2 appears to derive from rglNSCs themselves. Brako et al used fluorescence activated cell sorting (FACS) to isolate of GFP+ rglNSCs and their IPC progeny from Sox2:GFP mice, and identified IGF2 as highly expressed in these cells. *In situ* hybridization and antibody staining in the adult mouse hippocampus confirmed IGF2 expression was unique to DG, but not SVZ, NSCs and IPCs (Bracko et al., 2012). IGF2 protein was also detected in cultured rglNSC conditioned media by ELISA, further confirming IGF2 as part of the rglNSC secretome. IGF2 knockdown with a lentivirus expressing a short hairpin RNA against *Igf2* resulted in significantly decreased proliferation of infected cells both in the adult hippocampus and in cultured hippocampal rglNSCs but had no effect in SVZ NSCs *in vitro* or *in vivo* (Bracko et al., 2012). IGF2 was shown to signal through IGFR2 expressed in cultured DG rglNSCs, possibly stimulating proliferation by activating the AKT pathway. These data strongly suggest IGF2 is a DG rglNSC-specific, self-secreted factor that supports rglNSC maintenance, further establishing heterogeneity between the regional NSC secretomes of the adult brain.

MFGE8 is another soluble protein found within the adult DG rglNSC secretome that signals in an autocrine manner to maintain the stem cell pool (Zhou et al., 2018). Using transcriptional single cell RNAseq data, Zhou and colleagues found that MFGE8, a phagocytic factor, was enriched in DG rglNSCs and further investigation with antibody staining revealed its expression in astrocytes as well, but not IPCs or microglia. They found that knockdown of NSC-specific MFGE8 led exhaustion of rglNSCs, demonstrating a cell-specific role for NSC-derived MFGE8 despite production by local astrocytes (Zhou et al., 2018). The effect of MFGE8 appeared to rely on signaling through self-expressed integrin receptors, rather than any effect on phagocytic activity, suggesting that autocrine MFGE8 signals non-canonically to regulate the adult rglDG NSC pool. The role of MFGE8 in the SVZ type B NSC pool has yet to be established.

Similar to its role in development, SHH expression in adulthood may act as an autocrine signaling factor to maintain NSC pools. SHH is detectable in NSCs and IPCs in both adult neurogenic niches (Favaro et al., 2009). In the DG, SHH expression is closely tied to expression of the transcription factor SOX2. Conditional SOX2 knockdown reduced SHH expression in hippocampal rglNSCs, resulting in a significant reduction of rglNSCs and their progeny after 12 days, suggesting that SOX2 and SHH are necessary for maintaining the rglNSC pool (Favaro et al., 2009). Cultured SOX2 deficient DG-derived neurospheres exhibited impaired rglNSC maintenance, which was rescued by treatment with wildtype rglNSC conditioned media and inducible SHH expression (Favaro et al., 2009). Taken together, these data strongly suggest that autocrine SHH in the NSC secretome is necessary to maintain the DG rglNSC pool. In the SVZ, SHH functions similarly, where its expression is necessary to maintain NSC quiescence. Daynac and colleagues found that over-expression of NSC SHH increased NSC quiescence and downregulated neurogenesis. They further report that cultured adult SVZ NSCs respond similarly to exogenous SHH treatment (Daynac et al., 2016), suggesting that SHH also maintains the SVZ NSC pool. These discoveries of SHH and its role in autocrine signaling within

adult NSC pools suggest that some NSC secretome functions are conserved through development and across different neurogenic niches.

Similar to VEGF, WNT is a pleiotropic signaling molecule with numerous functions throughout the lifespan. In the developing CNS, WNT is primarily a niche-derived signal that supports neurogenic processes from rgNSC self-renewal to progenitor differentiation (Arredondo et al., 2020). WNT secretion has also been detected in cultured adult rat DG NSCs (Wexler et al., 2009), where blocking self-derived WNT signaling by overexpressing an N-cadherin fragment or axin to antagonize ß catenin led to NSC activation and proliferation. Similar to cultured adult DG NSCs, rglNSCs *in vivo* respond to WNT via canonical ß catenin signaling, which promotes balanced NSC quiescence and IPC proliferation. Interestingly, the *in vivo* DG niche also brings another potential interacting component in to WNT regulation of rglNSCs. Hippocampal IPCs express dickkopf-related protein 1 (DKK1), a WNT inhibitor (Niehrs, 2006), and NSC/IPC-specific DKK knockdown in adult mice drastically increased rglNSC activation (Seib et al., 2013). These findings suggest that balancing WNT exposure is a necessary step to ensure proper regulation of the adult hippocampal rglNSC pool. The potential cross-talk of IPC-derived DKK1 and rglNSC-derived WNT also exemplifies how autocrine regulation may still involve communication with neighboring cells, in this case between stem cells and progenitors within the niche. WNT expression was not found to be a prominent feature of SVZ type B NSCs, suggesting that this secretome-mediated self-maintenance mechanism, like that of VEGF, IGF2 and MFGE8, is unique to hippocampal NSCs.

Cystatin C is a secreted protein expressed by all nucleated cells but is most widely studied as a biomarker of kidney function (Onopiuk et al., 2015). In adult DG rglNSCs, the glycosylated form of Cystatin C (CCg) may act as a self-produced cofactor to support neurogenesis. For example, Taupin and colleagues found that supplementation of low density NSCs with conditioned media from high density NSCs resulted in rescue of the low density cells to sustain normal proliferation, suggesting that a NSC secreted factor was necessary for the survival of NSCs (Taupin et al., 2000). CCg was identified as a factor within the NSC conditioned media by mass spectrometry and its addition, along with the mitogen FGF2, to NSCs plated at clonal density supported proliferation and differentiation (Taupin et al., 2000). In the adult rat hippocampus, antibody staining and *in situ* hybridization confirmed Cystatin C was expressed in rglNSCs and IPCs. Cystatin C deficient mice had decreased adult hippocampal neurogenesis, however, as these mice were Cystatin C deficient across life in all cells the impact of Cystatin C derived specifically from rglNSCs in adulthood remains unclear. The authors confirmed that Cystatin C glycosylation promotes neurogenesis by grafting NSCs into the adult mouse DG alone, with FGF2 and CCg supplementation, or with FGF2 and a glycosylation deficient form of Cystatin C. They found that FGF2 and CCg were both required to stimulate neurogenesis in grafted tissue (Taupin et al., 2000). These findings highlight the potential of synergistic activity between factors in the NSC secretome and external niche factors like FGF2, much as the interaction between IPC-derived DKK and rglNSC-derived WNT does.

SDNSF, also known as multiple coagulation factor deficiency protein 2, is a soluble protein originally observed in adult rat hippocampal NSCs and is one of the few known secretome factors that is almost exclusively derived from stem cells *in vivo*, as opposed to being derived from stem cells as well as other cell types (Toda et al., 2003). SDNSF also appears to be specific to DG rglNSCs as *in situ* hybridization revealed SDNSF expression within DG rglNSCs, but not SVZ type B NSCs (Toda et al., 2003). Treating cultured DG NSCs from the adult rat hippocampus with recombinant SDNSF increased NSC survival, but not proliferation, after FGF2 withdrawal, suggesting that self-secreted SDNSF maintains DG rglNSC viability. The autocrine role of SDNSF also suggests that the NSC secretome may contain factors unique to stem cell populations that support the NSC pool or possibly their microenvironment.

Evidence from both the DG and SVZ suggests that NSCs may self-regulate their own response to ambient neurotransmitters via their secretome. In the adult SVZ, *in vivo* antibody staining revealed that type B NSCs and type C IPCs express diazepam binding inhibitor (DBI), a secreted, soluble molecule that can competitively bind to GABA receptors (Alfonso et al., 2012). SVZ type B NSCs and type C IPCs both express GABA receptors and are well established to decrease their proliferation in

response to GABA (Fernando et al., 2011; Liu et al., 2005). Alfonso et al., showed that overexpression of DBI in SVZ type B NSCs and type C IPCs increased their proliferation and subsequent neurogenesis, indicating that NSC/IPC-derived DBI may block GABA signaling and upregulate their own activation. Interestingly, SVZ neuroblasts spontaneously release GABA, which activates functional $GABA_A$ receptors on NSCs and IPCs, inhibiting proliferation and neural differentiation (Fernando et al., 2011; Liu et al., 2005). These findings suggest further cross-talk between the SVZ NSC secretome and that of their progenitors. In the adult hippocampal neurogenic niche, a similar expression pattern of GABA receptors has been observed. DG NSCs express the $GABA_A$ receptor, which is responsive to extracellular GABA (Song et al., 2012), possibly from synaptic GABA spillover (Farrant and Nusser, 2005). Similar to the SVZ, *in vivo* antibody staining suggests rglNSCs and DG IPCs express DBI and overexpression of DG NSPC-specific DBI induced rglNSC symmetrical division, bolstering the rglNSC pool (Dumitru et al., 2017). These findings agree with previous investigations into GABAergic signaling within DG rglNSCs, which showed that GABA promotes quiescence in DG rglNSCs, and GABA inhibition increases rglNSC activation and symmetrical division (Song et al., 2012). GABAergic inputs also promote neuronal differentiation, possibly by increasing calcium signaling in IPCs and increasing the expression of NeuroD (Tozuka et al., 2005). These studies together identify a unique mechanism within both adult neurogenic niches, whereby NSC-secreted factors may modulate their own response to neurotransmitters secreted by other cells, including in some cases their own progeny.

2.3.3 Adult NSC secretome: paracrine regulation, other factors and open questions.

Paracrine effects of the endogenous adult NSC secretome are far less well understood than the autocrine effects. One recent study showed that rglNSCs in the adult DG may regulate the maturation of their own progeny via secretion of pleiotrophin (PTN) (Tang et al., 2019). PTN is a pleiotropic soluble growth factor with numerous effects across the body. Tang and colleagues found PTN was specifically expressed by rglNSCs and IPCs in the SGZ and that PTN knockdown with a lentivirus expressing a short hairpin RNA against *Ptn* significantly reduced dendritic length and complexity. PTN is also expressed by SVZ NSCs (Qin et al., 2017), yet its role in paracrine signal to niche cells has yet to be established. These data strongly suggested a paracrine role of rglNSC secreted PTN in immature neuron maturation.

Beyond PTN, there is little research available on the paracrine functions of the adult NSC secretome. Multiple cell types express receptors for known NSC-derived, secreted molecules like WNT, VEGF, and IGF, suggesting the possibility for paracrine regulation of niche neighboring cells by NSCs. However, this possibility is largely uninvestigated to date. Paracrine function of NSCs in adulthood has been more commonly studied in disease models, particularly in terms of the ability of NSC transplants or conditioned media transfers to reduce neuroinflammtory response in injured CNS tissue. These studies suggest that adult and/or embryonic NSCs secrete products that are able to modulate the innate immune system and provide a paracrine benefit of transplant that is independent of engraftment of differentiated progeny. More in-depth reviews of this topic can be found in (Vogel et al., 2018; Willis et al., 2020a).

Understanding of both the autocrine and paracrine effects of the adult NSC secretome are currently limited by the rarity of exploratory studies to define the adult NSC secretome. One early study seeking to define the adult rodent NSC secretome performed two-dimensional electrophoresis and mass spectrometry on cultured NSCs from the adult rat hippocampus (Dahl et al., 2003). They identified several detectable proteins related to cell-to-cell communication, protein folding, membrane biogenesis, lipid transfer and cytoskeleton modification. This was one of the first examples of successful proteomic analysis to identify factors within the adult NSC secretome. More recently, we performed a mass spectrometry-based assessment of conditioned media from cultured adult hippocampal NSCs from mice (Denninger et al., 2020). We found NSC secretome proteins related to the positive regulation of gene expression, cell metabolism, IL-7 response, IGF binding, extracellular matrix modification and, similarly to Dahl and colleagues, cytoskeleton modification. Taken together, these studies suggest a rich NSC-secretome with many potential functions that remain to be investigated *in vivo*.

In summary, several key factors of the adult NSC secretome have been identified, most of which have been tied to self-regulation of adult neurogenic processes from NSC preservation to IPC differentiation (Figure 1). NSC autocrine factors appear to be anatomically and ontologically-specific with little overlap between adult SVZ, adult SGZ and prenatal NSC-regulating factors being evident in studies to date. Paracrine regulation of niche function by adult NSCs also seems likely but so far has not been extensively studied in endogenous, uninjured niches. Future studies focusing on *in vivo* identification of putative components of the NSC secretome and more directly investigating the NSC secretome impact other niche cell types, such as astrocytes, neurons, endothelia and microglia, are necessary to fully elucidate how NSCs may regulate their microenvironment under normal, healthy conditions.

2.4 NSC secretome in the aging brain

While neurogenesis persists across the rodent lifespan, the production of adult-born neurons drops precipitously in old age (Kempermann et al., 1998; Kuhn et al., 1996). Studies of the NSC pools and neurogenic niches of the aged brain thus far have focused mainly on mechanisms to bolster neurogenesis and thereby prevent cognitive decline in old age and disease models (Babcock et al., 2021; Lauretta et al., 2021; Maharjan et al., 2020). Little attention has been given to the NSC secretome in aging. Given the potent role of the NSC secretome in maintaining NSC pools during young adulthood, it seems likely that exhaustion of NSCs and decline of neurogenesis in the aged brain could be caused or exacerbated, at least in part, by changes in the NSC secretome. For instance, expression of numerous soluble factors known to derive in part from NSCs, such as WNT and VEGF, significantly decrease in the neurogenic niche with age (Palomer et al., 2019; Shetty et al., 2005). It is possible that a decline in the number of NSCs or in the quantity or quality of what the remaining NSCs secrete may contribute this effect, and thereby contribute to exhaustion of neurogenesis. To the best of our knowledge, though, there is a general dearth of investigations of the endogenous NSC secretome from the SVZ or SGZ of aged mice. This lack of studies investigating the aging NSC secretome may be due to their low number and detection limits of commonly used tools in proteomics. In the future, it is possible that this could be combated by the development of methodologies to more robustly derive NSCs from the aged brain, or more sensitive tools to detect secreted proteins from aged NSCs.

Though the aging NSC secretome is generally understudied, there is one prominent study from hypothalamic NSCs (htNSCs) which suggests the aging NSC secretome may be functionally relevant. Though the SGZ and SVZ host the vast majority of known NSCs in the adult mammalian CNS, htNSCs are also present in the rodent mediobasal hypothalamic region, a brain region important for regulating homeostasis. The mediobasal hypothalamus also plays a significant role in organismal aging (Zhang et al., 2017). Zhang et al found that htNSCs significantly decline with age, and depletion of the htNSC pool using a genetic model to ablate NSCs significantly accelerated the organism-wide aging process as defined by impaired locomotion, muscle endurance, coordination, sociability and cognition. The authors then showed that cultured htNSCs secrete exosomal miRNAs that were able to stimulate htNSC survival, improve both the physical and cognitive sequela of aging described above, and increase the lifespan when transplanted back into the hypothalamus of old mice (Zhang et al., 2017). While they do not identify specific miRNAs necessary to prevent/combat aging, this is one of the first and only studies to investigate the NSC secretome in the process of normal aging. However, as with the findings showing NSC-EV mediated changes in microglial activity during development, most of the supporting data rely on concentrated exosomes from cultured htNSCs. The function of physiological levels of htNSC-produced exosomes remains a target for future investigation.

3. Neural Stem Cell Secretome Across Species

3.1 The Human NSC Secretome

While most studies of the NSC secretome use rodent models, evidence is building to support the importance of the NSC secretome in humans. Neurodevelopment is highly conserved across mammalian species. rgNSCs in the developing human ventricular zone have bipolar processes which eventually serve as a migration scaffold for progenitors during cortical neurogenesis, much like rodents

do. In humans, rgNSCs begin neurogenesis around gestational week (gw) 5 and continue until gw 20 (deAzevedo et al., 2003; Rakic, 2004). After 20 weeks, the bulk of neuron production has occurred in the developing brain, and rgNSCs switch to gliogenic divisions (Zecevic et al., 2005). These pre and peri-natal events in human neurodevelopment have many parallels in rodents. As postnatal development proceeds, though, olfactory bulb neurogenesis derived from NSCs in the SVZ is not maintained in humans (Bergmann et al., 2012; Sorrells et al., 2018; Wang et al., 2014). In contrast, neurogenic NSCs do appear to be maintained in the adult human hippocampus (Figure 2), though this is currently controversial. A preponderance of data support the maintenance of hippocampal neurogenesis throughout life in humans (Boldrini et al., 2018; Dennis et al., 2016; Eriksson et al., 1998; Moreno-Jiménez et al., 2019; Spalding et al., 2013; Tobin et al., 2019), as well as specific preservation of NSCs well into human aging (Mathews et al., 2017). However, one key study recently presented findings to the contrary (Sorrells et al., 2018), leading to a debate about the very existence of adult hippocampal neurogenesis in humans and the proper methods for addressing that question. As this debate highlights, studying endogenous NSCs in humans is technically challenging. Because of these challenges, relatively few studies have addressed the function of the endogenous NSC secretome in humans. The focus of NSC secretome studies in humans has instead thus far been on cultured NSCs.

Understanding of the human NSC secretome within the healthy brain is currently limited by the rarity of exploratory studies to define it. Only a small number of studies have attempted an unbiased characterization of the NSC secretome, exclusively using conditioned media from cultured NSCs. Recently, Cervenka et al., performed mass-spectrometry on conditioned media from cultured human NSCs differentiated from embryonic stem cells *in vitro*. They detected the secretion of 28 growth factors and cytokines, including VEGF and IL-6 (Červenka et al., 2020). Another study of cultured human embryonic cortex NSC-produced exosomes focused on their secretion of miRNAs (Stevanato et al., 2016). They found that human NSC-produced exosomes highly expressed miRNA-1246, 4488, 4508 and 4516, which can have a wide range of effects including the regulation of cell growth and apoptosis. While these studies have begun to define the human NSC secretome, there is a clear need for future investigations to determine what other factors are present, such as the morphogens, growth factors, cytokines, chemokines and other soluble proteins that have been identified in the rodent NSC secretome.

Several studies of the human NSC secretome have taken a more targeted approach of searching for pre-selected factors in the NSC secretome then testing their potential therapeutic relevance in models of disease. Most studies use cultured human NSCs derived from differentiated human embryonic NSCs or from fetal brain tissue and test their function by infusion of conditioned media or whole NSC transplant into mouse models of disease. For example, Lee et al., used western blot to identify the expression of trophic factors relevant to Alzheimer's disease in the conditioned media of cultured human NSCs. They found that human NSCs isolated from 13 week old fetal brain tissue secreted brain derived neurotrophic factor (BDNF), nerve growth factor (NGF), VEGF, FGF2, and glial cell line-derived neurotrophic factor (GDNF) (Lee et al., 2015). Transplant of human NSCs into a mouse model of Alzheimer's disease increased synaptic density, reduced apoptosis, and reduced amyloid β load, all improvements in the host tissue that the authors attribute to the NSC secretome (Lee et al., 2015). Several other studies similarly support the therapeutic role of the human NSC secretome in stroke (Eckert et al., 2015; Hicks et al., 2013; Huang et al., 2014). For example, intracerebral infusion of conditioned media from cultured embryonic cortex-derived human NSCs—which contained factors such as IGF1, VEGF, basic FGF and EGF—led to improvement in blood vessel remodeling, sprouting and angiogenesis in a mouse model of ischemic stroke (Hicks et al., 2013). Multiple sclerosis has also been shown to be particularly susceptible to the anti-inflammatory properties of the human NSC secretome. Reduction in neuroinflammation, enhanced remyelination and improved motor behavior have all been linked to treatment with human NSC-derived conditioned media containing factors such as TGFβ (Aharonowiz et al., 2008; Chen et al., 2014; Pluchino et al., 2009). While these disease-focused studies suggest that the human NSC secretome has potent functional roles in the CNS, the function of endogenous human NSC secreted products, in healthy or diseased brains, remains largely uninvestigated.

While studies of the human NSC secretome are limited, the apparent conservation of many secretome factors between rodent and human NSCs gives hope that the rodent literature will be predictive for humans. For example, overlapping secretome factors in cultured human ESC-derived NSCs and rodent embryonic rgNSCs include VEGF, IL-6, and miRNAs. In rodents, these factors induce endothelial ingression into the developing CNS, regulate rgNSC quiescence and influence microglia morphology and physiology, raising the possibility for similar function in human rgNSCs during neurodevelopment. Similarly, the production of VEGF by cultured human ESC-derived NSCs overlaps with the secretome of adult rodent hippocampal or SVZ NSCs. In rodents, these factors regulate NSC quiescence, suggesting the potential for a functional autocrine role of the human NSC secretome. These possibilities suggested by parallels with rodents remain open for future investigation.

3.2 The Zebrafish NSC Secretome

Investigation of the NSC secretome is not limited to mammalian species. Zebrafish, and other aquatic vertebrates, have established NSC niches which contribute to robust lifelong neurogenesis. Similarly to mammals, zebrafish radial glia (RG) arise during CNS development, are the origin for extensive developmental neurogenesis, and express many markers related to NSCs including Sox2, GLAST, Nestin and GFAP (Berberoglu et al., 2014; März et al., 2010). Unlike rodents, zebrafish RGs are widely maintained in the adult CNS and do not transition or mature into a unique cell population after CNS development (Than-Trong and Bally-Cuif, 2015). RGs are active in many neurogenic niches in the adult zebrafish CNS including the pallium, lining the ventricles, the hypothalamus, the tectum and cerebellum. Neurogenesis in these distinct subregions occurs at different rates (Grandel et al., 2006). Similarly to NSCs in adult rodents, zebrafish RGs produce new neurons constitutively and are reactive to insults including stab wounds and exposure to toxins, which greatly enhance neurogenesis in different CNS subregions (Kizil et al., 2012; Schmidt et al., 2013; Skaggs et al., 2014). The balance of RG quiescence and activation is also regulated by similar mechanisms as rodent NSCs, including the Notch, WNT and IGF pathways (Borday et al., 2012; Chapouton et al., 2010; Wan et al., 2014). Taken together, these molecular and cellular similarities suggest that zebrafish may provide a relevant model for insight into mammalian NSC biology.

Understanding of the zebrafish RG secretome in the healthy CNS is less advanced than that of rodent and human NSCs. In 2019, Obermann and colleagues published the first broad investigation into the zebrafish RG secretome. They examined the proteome associated with the cell surface of freshly isolated zebrafish telencephalic RGs (Figure 2) with mass spectrometry and found 557 proteins associated with the formation of extracellular exosomes. Interestingly, approximately 10% of the proteins they identified were associated with secretion for intercellular signaling (Obermann et al., 2019). This study suggests that zebrafish RGs may regulate themselves and surrounding tissue via their secretome, much as mammalian NSCs do. Harnessing of the zebrafish model for advancing understanding of NSC secretomes remains an open avenue for future studies.

3.3 NSC Secretome in Other Species

Beyond mammals and fish, neurogenic niches can also be found throughout life in birds, reptiles, and amphibians (Alunni and Bally-Cuif, 2016), suggesting that many properties of NSCs may be evolutionarily conserved. Across these species, the functions of NSCs in developmental neurogenesis and the production of adult-born neurons are well established. However, there is a dearth of studies investigating the NSC secretome in these other model systems. The roles of WNT, BMP and SHH signaling are highly conserved during CNS development (Ashe, 2016; Letelier et al., 2018; Steinhart and Angers, 2018) and growth factors, like VEGF and IGF, are known to stimulate neurogenesis across species (Barbieri et al., 2003; Chen et al., 2013; Erkenbrack and Petsios, 2017; Louissaint et al., 2002). Whether the NSC secretome plays a role in stimulating that signaling across species remains unclear. Paracrine signaling by NSCs across species also remains almost completely uninvestigated to our knowledge. Future studies of the NSC secretome from these species may reveal new insight into the endogenous NSC secretome and its function within the healthy CNS.

4. Neural Stem Cell Secretome in Disease

Investigation into the clinical applications of transplanted NSCs have revealed potential for the NSC secretome in regenerative medicine. The transplanted NSC secretome has been shown to exert therapeutic effects through three major mechanisms: stimulating neuroprotection, enhancing CNS plasticity, and regulating the neuroimmune system (Zhang et al., 2020). Cultured NSCs from varying sources can secrete factors, such as VEGF, GDNF, IGF and BDNF, that improve host neuron survival following NSC transplant or treatment with NSC conditioned media after spinal cord injury, Parkinson's disease, traumatic brain injury and Alzheimer's disease (Hu et al., 2020; Lee et al., 2015; Mendes-Pinheiro et al., 2018; Ziv et al., 2006). Cultured NSCs also can increase CNS plasticity through the secretion of GDNF, FGF, VEGF and other factors which stimulate dendritic growth, axonal regeneration, and angiogenesis in models of spinal cord injury and ischemia (Hicks et al., 2013; Mendes-Pinheiro et al., 2018; Romanyuk et al., 2015). Modulation of the neuroimmune system is also a prominent feature of exogenous NSC secretome products. Through the secretion of TGFß and anti-inflammatory cytokines, transplanted NSCs have been shown to decrease neuroinflammation and inhibit the activation of immune cells in models of ischemia, multiple sclerosis and spinal cord injury (Chen et al., 2014; Rong et al., 2019; Yang et al., 2018). These findings establish the therapeutic potential of NSC secreted factors derived from exogenous NSCs and are reviewed more thoroughly in the following sources (Willis et al., 2020b, 2020a; Zhang et al., 2020). Whether these functions of exogenous NSCs mimic those of endogenous cells during injury or diseases remains unclear. Many CNS insults including traumatic brain injury, ischemia and Alzheimer's disease alter neurogenesis, suggesting that they impact endogenous NSCs. Furthermore, manipulations of endogenous NSC pools to increase growth factor expression has been shown to enhance recovery in many disease pathologies (Ohori et al., 2006; Song et al., 2014; Sun et al., 2020). However, there are a lack of studies investigating how the endogenous NSC secretome influences pathology or recovery in disease states. This is a relevant open question for any effort to support CNS recovery via manipulation of the endogenous NSC population. More investigation of the endogenous NSC secretome following CNS injury and disease is therefore needed to clarify the role of these cells in disease pathology and recovery.

5. Conclusion

Research to date supports autocrine, paracrine and bidirectional signaling roles of the NSC secretome across neurodevelopment in multiple species. NSC secreted factors have been most studied during prenatal development and in adulthood for their autocrine regulation of the NSC pool in rodents. Much less is known about the NSC secretome in the juvenile and aging brain or its role in paracrine signaling to niche cells under physiological conditions. We found no studies examining the endogenous NSC secretome following NSC injury or disease. Our comparison across species revealed conservation of multiple secretome factors between rodents and humans, supporting the applicability of studying the NSC secretome in model organisms. Other species with more abundant NSC niches in adulthood, such as zebrafish, have received less attention but may offer fruitful avenues for future study of NSC secretome interaction with mature CNS tissues. Despite an incomplete understanding of how NSCs interact with their niche, they are being actively pursued as a therapeutic (Alia et al., 2019; McAvoy and Sahay, 2017)(Alia et al., 2019; McAvoy and Sahay, 2017), including through ongoing human clinical trials using NSC transplants (Willis et al., 2020b). A more thorough understanding of how the endogenous NSC secretome influences the surrounding niche is still needed to help guide these attempts to harness NSCs for therapeutic purposes, as well as to advance fundamental understanding of NSC functions in the CNS.

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