PERSPECTIVE

Insights into ligand expression heterogeneity across multiple cell types in the adult forebrain that regulates neural stem cell behavior

In the adult mammalian central nervous system (CNS), neural cell generation is restricted to two highly specialized germinal niches. These are the subventricular zone (SVZ) that borders the forebrain lateral ventricles and the subgranular zone of the hippocampus. In the case of the former niche, the SVZ gives rise daily to thousands of migratory neuroblasts that travel long distances to the olfactory bulb via the rostral migratory stream. Neural stem cells (NSCs) first engage through a sequence of activation phenotypes and give rise to transiently amplifying progenitors (TAPs) that subsequently differentiate into neuroblasts. Once in the olfactory bulb, they generate phenotypically distinct neurons. Most of these constitute the interneuron class that is preferentially derived from the lateral portion of the SVZ, whilst a small proportion of projection neurons are also formed from the dorsal SVZ. Similarly, although fractional in number, oligodendrocyte lineage cells and astrocytes are additionally generated from this germinal zone. Lesser is known regarding the differentiation steps regarding adult astrogenesis from the SVZ. Oligodendrocyte lineage cells diverge from TAPs and remain relatively close within the periventricular forebrain corresponding to their site generation (**Figure 1**). Oligodendrocytes generated from the SVZ will also pass through phenotypically distinct stages until they form mature oligodendrocytes. Adult NG2 glia (oligodendrocyte precursor cells) are the main proliferative population of the adult CNS outside of neurogenic niches and are supported in their capacity to restore oligodendrocyte populations following injury by SVZ derived early oligodendroglial populations.

The SVZ is a thin tissue that surrounds a highly multifaceted ventricular system and is adjacent of the periventricular white matter in its dorsal aspect, the striatum in its lateral side and a medial portion neighbored by the septum. Each of these adjoining tissues is highly heterogeneous in their cell populations, thereby adding to the complexity of the macroenvironment. Numerous reports have evidenced that the walls of the adult SVZ are a continuation of germinal activity observed early during forebrain development, as they are additionally synchronized by their local environment (dorsal, lateral and medial) (Fiorelli et al., 2015; Azim et al., 2016). A number of mechanisms that regulate SVZ heterogeneity have been reported. These include and are not limited to, transcriptional control, epigenetic regulation, the extracellular matrix and most notably, the expression of secreted trophic factors. It is highly accepted that NSC subpopulations are primed in a time-controlled manner for the generation of neural subtypes that are ultimately regulated by the expression of transcription factors (TFs) that control cell fate. TF expression and activities will in a large extent rely on the presence of external stimuli that regulate NSC dynamic states and lineage progression. Key patterning ligands include canonical Wnts and Shh that maintain dorsal SVZ and ventral regionalization, respectively (Fiorelli et al., 2015; Azim et al., 2016; Azim et al., 2017), whose sources of origin are well known. Similarly, a number of ligands will maintain NSCs in a generic manner by providing them with trophic support, enhancing their proliferation or reverting them into quiescence. Therefore, understanding signaling-to-TF networks is critical for recruiting region-specific NSCs in the case of injury or trauma. In this regard, stimulating pathways that activate SVZ subpopulations of NSCs for generating specific lineages will be necessary for appropriate cell fate-specification. In order to address these, it is important to identify which are the appropriate environmental cues that NSCs are exposed to, in both normal states and during injury or disease.

A number of lines of investigation in the field have unraveled NSC biology, down to the single cell level. These studies have focused on NSCs that are present along the lateral portions of the SVZ, which in normal circumstances produce interneurons in abundance with fewer output of other neural lineages. However, differences in microdomain-specific NSCs and their local macroenvironment that support them is relatively unknown, which was the underlying aim in defining the expression of ligands in CNS compartments relative to neurogenesis/gliogenesis. In order to unwrap some of the complexities of ligand 'secretome', a meta-analysis was performed on numerous whole genome transcriptome datasets for multiple cell types that interact with the SVZ niche or form this germinal zone derived from the mouse forebrain. These included NSCs, TAPs, neuroblasts, ependymal cells, oligodendroglia, astrocytes, microglia, endothelia, pericytes and the choroid plexus. By analysis of publically available datasets we could define ligand signatures across multiple cell types that delineates to an extent the macroenvironment of the niche. Interestingly, over half of all ligands were detected in cells of the vasculature (59.5%), followed by subtypes of glial cells (18%), the choroid plexus (12.5%) and NSC substages that expressed relatively fewer ligands (10%). A number of ligands that amounted to approximately 13.5% across these four defined groups were expressed in multiple cell types. Likewise, ligands with similar functional properties (*i.e.* targeting similar downstream pathways or the same receptors) or from the same family were found to be homogeneously distributed in expression across different cell types. Good examples of these were, 'signaling *via* FGFR2', 'chemokines' and 'PIP3/Akt' signaling. This implies levels of redundancy among a large group of ligands. Most importantly, the vasculature was noted as a rich source of ligand mRNA, with endothelia expressing many transcripts of ligands that are able to induce differential effects onto NSCs/progenitors in the SVZ.

The large number of ligands detected in endothelia with specific expression to these cells and not from other sources is an interesting observation. Given the abundance of the vasculature of the brain, future studies analyzing the proteome of endothelia and pericytes from the SVZ microdomains would be able to verify ligands expressed at the mRNA level are indeed translated into proteins. The large numbers of patterning molecules, such as Wnts, BMPs/TGFβ and FGFs downstream target genes include many ligands themselves (Minami and Aird, 2005), and as such, their functional relevance to NSCs/progenitors would benefit from proteomic profiling studies. It is predicted that many of the abundant ligands expressed in endothelia reflect vascular-specific autocrine or paracrine control, and may not be directly affecting NSCs.

Ligands expressed in glial cells of the corpus callosum are more relevant to the dorsal SVZ, based on spatial proximity, which is highly intriguing. Little is known regarding the locally derived cues that regulate dorsal NSC maintenance and our work sheds some light. A recent study described that the young adult dorsal SVZ expresses markers that define dorsalization, including Emx1 (and Pax6) together with the Wnt-target genes (*Lef1, Tcf, Axin2*). These findings are summarized in heatplots of the SVZ microdomains (**Figure 2A–H**) that were derived from gene expression profiling experiments as described recently (Azim et al., 2017). Target genes such as *Stat1, Fos, Jun, Cyp26a1* and a number of others (unpublished observations) that are indicative of more general growth factors (fibroblast growth factors, insulin-like growth factors, neurotrophins) were highly enriched in the ventral SVZ. In addition to the choroid plexus secreting these ligands, their respective receptor's level of expression in the distinct microdomains may reflect the elevated expression of 'routine ligands' target gene expression. This implies that NSCs present in the distinctive microdomains respond differentially to ligands derived from distant sources, presumably by the expression of receptors in region-specific NSCs. Later in adulthood, markers of NSC activation in the dorsal SVZ decline dramatically as per the expression of Wnt-target genes. On the other hand, the ventral SVZ during the later stages of adulthood continue to express genes related to general growth factors, including *Gli1* that is highly expressed upon Shh signalling. Interestingly, genes that are used as readouts for both BMP/TGFβ-signalling and NSC quiescence included at least *Id1-4* amongst others, indicating that these types of ligands may be locally derived from

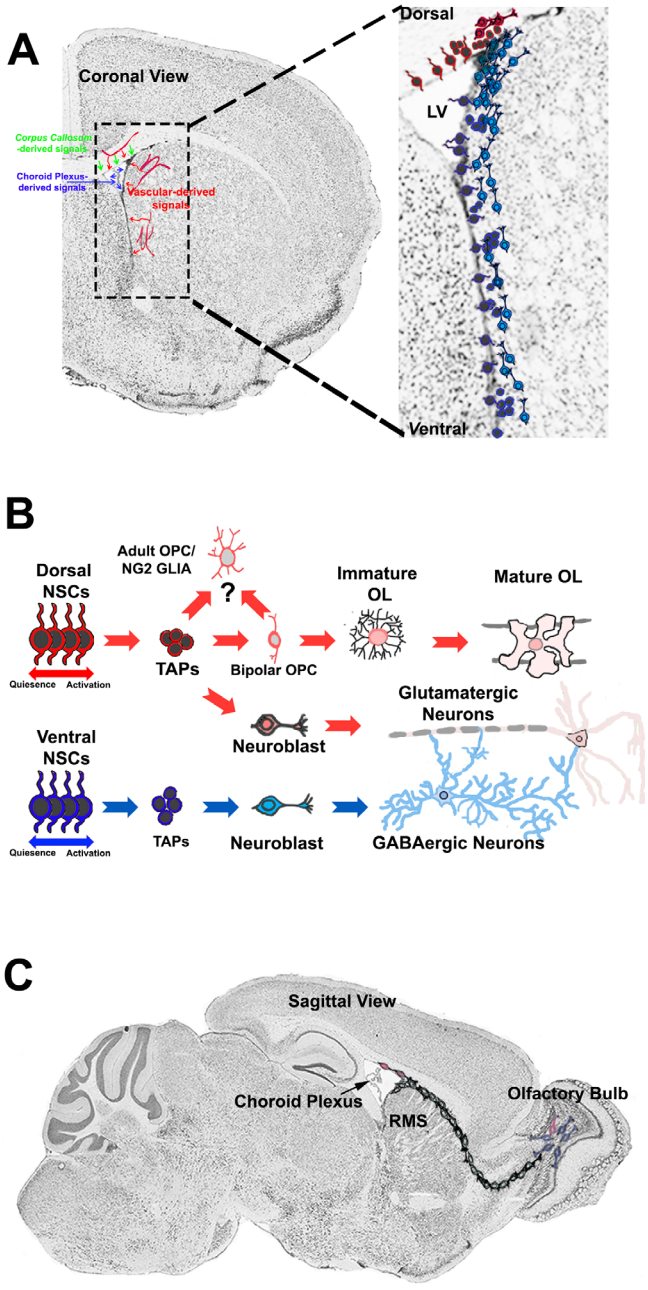

Figure 1 Neurogenesis/oligodendrogenesis in the young adult SVZ of the mouse forebrain.

(A) An overview of a coronal section of the forebrain, highlighting the SVZ and its relationship to adjacent structures that provide trophic support. The SVZ is expanded and schematically shown the major neurogenic compositions of the niche. Dorsally located NSCs are colored in red, whereas those located along the lateral wall are in blue. (B) Differentiation steps of region-specific NSCs. Dorsal NSCs are present in different states of differentiation (*i.e.,* quiescent, primed, activated), and upon their activation generate TAPs. Some TAPs will differentiate into OL lineage cells with the potential to generate fully myelinating OLs or remain in the parenchyma as adult NG2 glia. Dorsal SVZ-derived TAPs will also generate neuroblasts that eventually form glutamatergic neurons. The most numerous NSCs, the ventral (or lateral) NSCs generate large numbers of GABAergic interneurons *via* TAPs and neuroblasts. (C) Neuroblasts formed in the SVZ will migrate long distances *via* the RMS into the olfactory bulb. LV: Lateral ventricle; NSCs: neural stem cells; OL: oligodendrocyte; OPC: oligodendrocyte precursor cell; RMS: rostral migratory stream; TAPs: transiently amplifying progenitors. SVZ: subventricular zone; GABA: gamma-aminobutyric acid; NG2: nerve-glia antigen 2.

adjacent glial cells of the corpus callosum that counteract dorsal NSC-activation over time. This is supported by the down-regulation of routine ligands (*i.e.* growth factors such as fibroblast growth factor 2) as described above that are known to promote proliferation and lineage progression.

The main findings are summarized in **Figure 2**. Additionally some of the key ligands that are abundantly expressed in oligodendroglia and astrocytes are highlighted in **Figure 2I** and **J**. Some of these ligands correlate with the expression of transcripts in the dorsal SVZ. For example ligands such as *Bmp1/Bmp4* amongst others, whose target genes include members of the Id family of TFs, may promote quiescence and these ligands increase in expression in oligodendroglia in later adulthood. Similarly, astrocyte derived *Wnt7b*, despite an elevated expression, together with *Dkk3* may fine-tune tightly NSC activities in the dorsal wall. The decline in dorsal SVZ neurogenic activities in ageing correlates with higher expressions in *Dkk3* as described elsewhere and is in agreement with our own observations (Zhu et al., 2014).

This is at least one clear example of identification of pathways and ligands relevant to specific dorsal microdomains and its niche that could be probed for testing of regeneration and rejuvination. In the latter instance, activating Wnt-signaling pharmacologically in periods of adulthood where the dorsal SVZ activities are relatively minimal compared to earlier periods, massively promotes dorsal lineages and rejuvenates this niche (Azim et al., 2017). A number of other highly expressed glial-derived ligands such as *Edil3, Chrdl1* (**Figure 2I** and **J**) amongst others are poorly understood and warrants further studies. It is also very interesting that glial cells close to the dorsal wall may secrete cues to regulate NSC behavior for neural turnover. How and why glial cells close to the walls of the SVZ secrete cues and what are the upstream players remain to be identified.

There are at least a few implications for the study (Azim et al., 2018): (1) The identification of numerous ligands across multiple cell types suggests that NSC processes are not linked to a handful of ligands only and it is very dynamic in nature. Depending on the level of receptor expression, multiple ligands will coordinate varying steps during NSC differentiation. One of the major ligands that are used for sustaining and expanding NSCs in culture conditions, *Egf*, was not detected *in vivo*. However, other similar ligands such as *Tgfa* that is highly expressed/abundant in oligodendrocytes (**Figure 2I**) may have different functions on NSCs. The similarities and differences between these two and other similar signaling ligands of the EGF family and the downstream pathways are not known. As our results suggest and as we proposed initially, regulation of neurogenesis will come from multiple ligands. This will be informative to the types of ligands that should be considered for future studies modeling the environment of NSCs in *ex vivo* preparations. (2) As described briefly above, the ever increasing datasets of purified cell types at the bulk or single cell level can help reveal which are the ligands that are temporally altered during adulthood or ageing. Future analyses can reveal inhibitory ligands that increase during ageing or ligands that disappear in expression that provide trophic support. These will provide additional therapeutic targets for ageing studies. (3) Comparing the expression profiles of healthy with diseased states of specific cell types will be very intriguing. For example, with the case of neuroinflammation, examination of microglia and astrocyte ligand expression signatures can define major signaling pathways altered that limit regeneration in the vicinity of these two cell types.

Further work is underway in examining ligands that are highly expressed in germinal zones, and their influence on different stages of NSC behavior and specification into different lineages. Other work is ongoing where glial cells have been isolated following injury. Comparison with healthy isolated cells as discussed here has already shed some light on the signaling environments. The work was performed in order to systematically probe the origins of ligands for major cell types that could regulate neurogenesis without the need to perform complicated bioinformatic analyses. It appears that in terms of what we know in the field regarding signaling mechanisms associated with SVZ-NSCs *in vivo*, is still evolving.

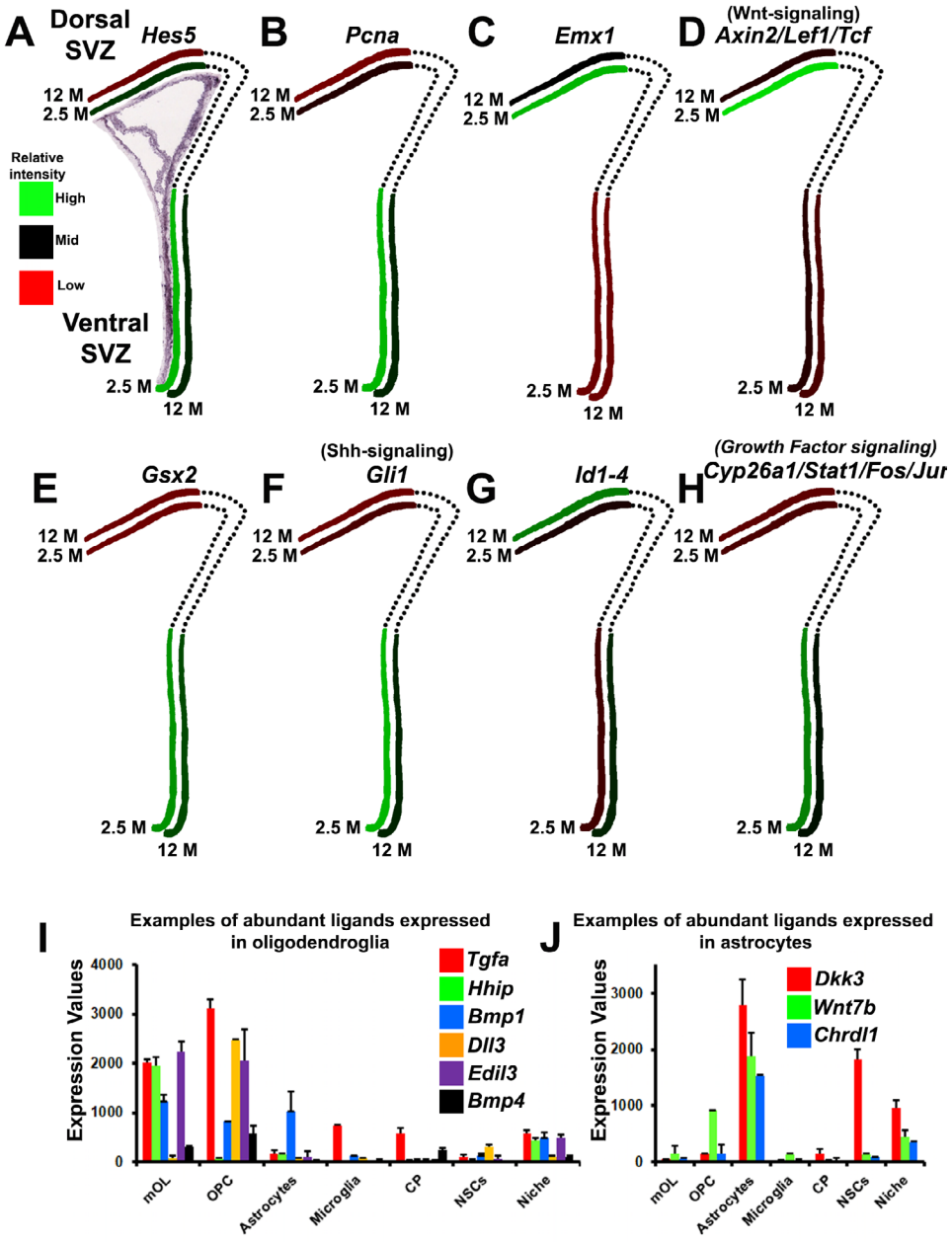

Figure 2 SVZ microdomain maintenance by signaling ligands.
(A–H) The walls of the micro-dissected adult mouse SVZ at 2 different time points (2.5 months (M) and 12 M of age) were sampled by qPCR as described previously (Azim et al., 2017). These findings are summarized as heat intensities for specific genes as readouts of signaling activities or markers of SVZ microdomains. *Hes5*: Notch signaling; *Pcna*: proliferation; *Emx1*: Dorsal NSCs; *Axin2/Lef1/Tcf*: Wnt pathway target genes; *Gsx2*: subpallial germinal zone marker; *Gli1*: Shh pathway target gene; *Id1–4*: target genes for BMP/TGFβ signaling and Notch signaling; *Cyp26a1/Stat1/Fos/Jun*: signaling pathway target gene markers for a number of routine growth factors. In the case of multiple genes where the expression intensities were similar, the average was selected. (I, J) The expression patterns of the most abundant ligands in oligodendroglia and astrocytes. Key ligands were selected that were highly enriched and significant in their respective cell type *versus* other cells (over 2.5 folds enrichment with false discovery rates of > 1%) (Azim et al., 2018). NSCs: Neural stem cells; mOL: mature oligodendrocyte; OPC: oligodendrocyte precursor cell; CP: choroid plexus; SVZ: subventricular zone.

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