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What has single-cell transcriptomics taught us about long non-coding RNAs in the ventricular-subventricular zone?

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SUMMARY

Long non-coding RNA (lncRNA) function is mediated by the process of transcription or through transcript-dependent associations with proteins or nucleic acids to control gene regulatory networks. Many lncRNAs are transcribed in the ventricular-subventricular zone (V-SVZ), a postnatal neural stem cell niche. lncRNAs in the V-SVZ are implicated in neurodevelopmental disorders, cancer, and brain disease, but their functions are poorly understood. V-SVZ neurogenesis capacity declines with age due to stem cell depletion and resistance to neural stem cell activation. Here we analyzed V-SVZ transcriptomics by pooling current single-cell RNA-seq data. They showed consistent lncRNA expression during stem cell activation, lineage progression, and aging. In conjunction with epigenetic and genetic data, we predicted V-SVZ lncRNAs that regulate stem cell activation and differentiation. Some of the lncRNAs validate known epigenetic mechanisms, but most remain uninvestigated. Our analysis points to several lncRNAs that likely participate in key aspects of V-SVZ stem cell activation and neurogenesis in health and disease.

INTRODUCTION

The ventricular-subventricular zone

The ventricular-subventricular zone (V-SVZ) (Figure 1) is a neurogenic stem cell niche that plays roles in replenishing olfactory bulb (OB) neurons throughout life in most mammals and in the response to brain injury (Altman, 1969; Chang et al., 2016; Young et al., 2011). This fascinating neurogenic niche has been reviewed extensively elsewhere (Ihrie and Alvarez-Buylla, 2011; Katsimpardi and Lledo, 2018), so we will only briefly introduce it. The neurogenic capacity of the V-SVZ declines with age, both due to depletion of neural stem cells (NSCs) and due to NSCs becoming increasingly resistant to activation (Kalamakis et al., 2019; Navarro Negredo et al., 2020). Quiescent NSCs in the V-SVZ become activated and generate intermediate transit amplifying progenitor cells (Doetsch et al., 1999). These sessile cells divide a limited number of times and give rise to neuroblasts, which migrate via the rostral migratory stream to the OB where they differentiate to form interneurons (Altman, 1969). NSC and neuroblast numbers can increase in injury (Szele and Chesselet, 1996) and emigrate to ectopic regions of disease and thereby mount a regenerative response (Chang et al., 2016). Whereas the adult V-SVZ is primarily neurogenic, demyelination signals the niche to generate oligodendrocyte precursors that emigrate and participate in remyelination. This recapitulates the postnatal V-SVZ, which in addition to being neurogenic is gliogenic and gives rise to large numbers of oligodendrocytes and astrocytes that populate the forebrain (Levison et al., 1993).

The techniques available for studying the human V-SVZ are limited, and the large majority of studies have only examined it in postmortem sections with immunohistochemistry (reviewed in Gault and Szele, 2021). Whereas this technique is plagued by problems of antigen over-fixation, variable postmortem delays in tissue collection, poor characterization of antibodies, etc., the large majority of studies have concluded that, both constitutively and after injury, there is evidence for adult human V-SVZ stem cells and neurogenesis but that postnatal neurogenesis is more robust than adult neurogenesis (Gault and Szele, 2021). As well, there is collective agreement that in humans, neurogenesis occurs during the first year of life (Sorrells et al., 2018). In order to completely confirm adult human neurogenesis, more techniques such as non-invasive imaging and cell culture will have to be adapted to humans. It was through a combination of intersecting techniques that adult neurogenesis in animals was proven. Already a small handful of birth-dating studies have suggested that adult human V-SVZ cells are neurogenic (Eriksson et al., 1998; Ernst et al., 2014). The study by Ernst and colleagues used carbon-14 birth-dating and found evidence that the adult human V-SVZ generates calretinin and NPY + interneurons in the striatum. If confirmed, this is important because significant reductions in striatal calretinin interneurons are found in autism spectrum

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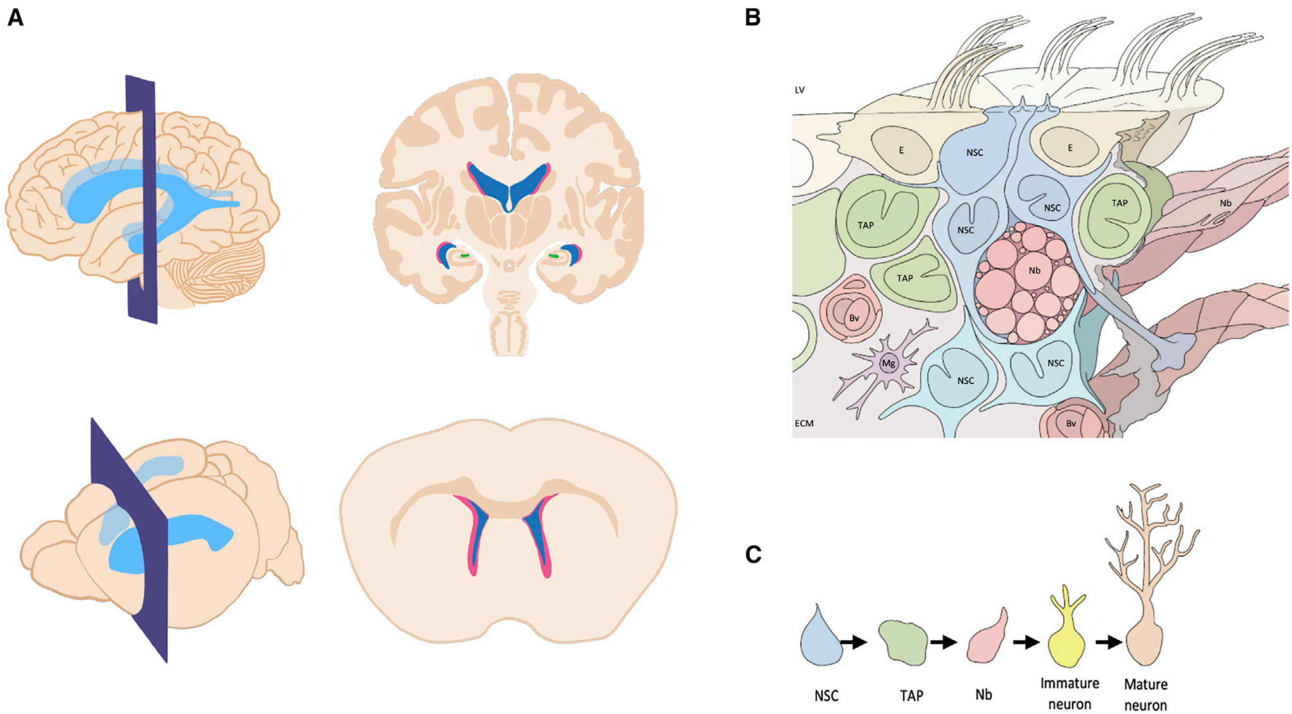

Figure 1. V-SVZ cytology and lineage progression

(A) Comparison of the lateral ventricle (blue) and V-SVZ (pink) in human (top) versus mouse (bottom). Left: see-through views showing the shape and location of the lateral ventricles from a sagittal perspective. Right: coronal sections.

(B) Cellular anatomy of the V-SVZ.

(C) Neurogenic lineage progression. Abbreviations: E, ependymal cell; NSC, neural stem cell; TAP, transit amplifying progenitor; Nb, neuroblast; Mg, microglial cell; Bv, blood vessel; ECM, extracellular matrix; LV, lateral ventricle. (B) adapted from Ihrie and Alvarez-Buylla (2011) with permission from Elsevier. (C) adapted with permission from Batiz et al. (2015).

disorder and schizophrenia (Adorjan et al., 2017; Adorjan et al., 2020).

Single-cell RNA sequencing (scRNA-seq) will thus be an essential tool to study adult human V-SVZ cells. This has already commenced with one study finding an inflammatory signature in the aging human V-SVZ (Bitar et al., 2022) and another suggesting loss of stem cell activation in the human niche could be rescued with Wnt signaling (Donega et al., 2022). Thus, the field generally remains hopeful of either increasing or decreasing these endogenous stem cells to treat disease, especially if the "right" diseases are targeted (Goldman, 2016). It is accepted that human gliomas can arise from the SVZ and the knowledge of which cells these cancers arise from is within reach (Bardella et al., 2016; Vescovi et al., 2006).

Despite our current knowledge of cell types and functions, many features of V-SVZ cells have remained mysterious until recently, with their study confounded by the numbers of spatially distinct and phenotypically dynamic cell types within the niche (Merkle et al., 2007; Mizrak et al., 2019). Most groups study the "striatal SVZ," which is in the lateral wall of the lateral ventricle. However, the "septal SVZ," which lines the medial wall of the lateral ventricle, also contains stem cells and is neurogenic and recently was shown to have significant sex differences in V-SVZ gene expression (Mizrak et al., 2019).

Another remarkable study showed that NSCs with reduced PDGFRb become activated and generate oligodendrocytes (Delgado et al., 2021). These oligodendrocyte progenitors are found in distinct subdomains on the inside of the ventricular wall (Delgado et al., 2021), and therefore they could easily be sloughed off during tissue preparation in scRNA-seq studies. Nevertheless, these cells are of special interest as they may respond to demyelinating injuries and also participate in gliomagenesis (Delgado et al., 2021). Additionally, significant dorsoventral differences have been noted, including persistence of radial glialike cells in the ventral most V-SVZ (Sundholm-Peters et al., 2004), sonic hedgehog affecting the ventral V-SVZ (Ihrie et al., 2011) and Wnt signaling primarily affecting the dorsal V-SVZ (Azim et al., 2014).,. Similarly, scRNA-seq has shown differential gene expression between dorsal and ventral V-SVZ (Cebrian-Silla et al., 2021). However, most other scRNA-seq studies do not specify the anatomical

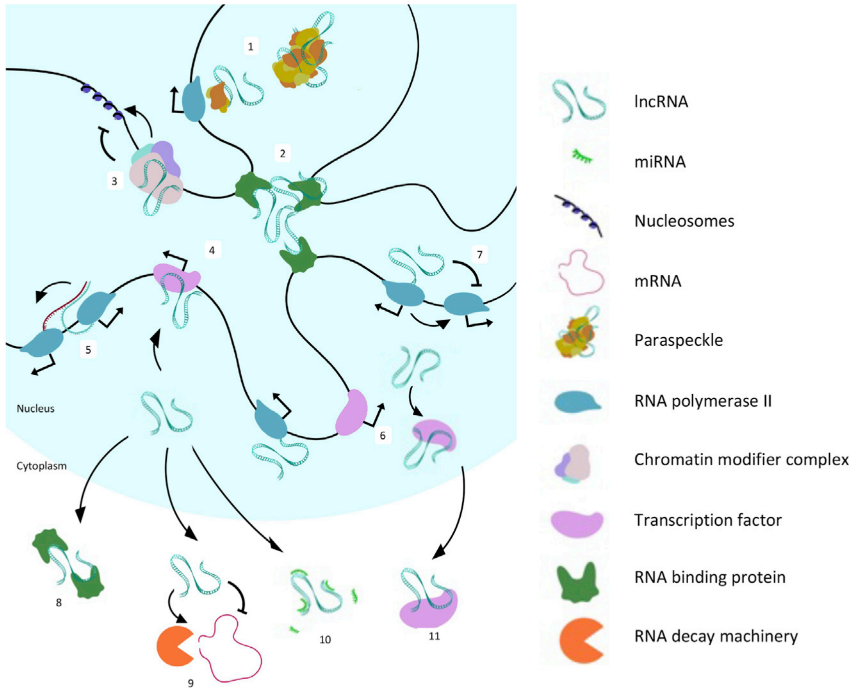

Figure 2. Some roles of lncRNAs in eukaryotic cells

(1) In formation of nuclear paraspeckles and other phase-separated domains. (2) In chromatin looping. (3) In regulation of chromatin-modifying complexes. (4) As a transcription factor guide. (5) In the formation of R-loops. (6) As a transcription factor decoy. (7) In transcriptional interference. (8) In control of RNA-binding protein localization. (9) In regulation of mRNA decay. (10) As miRNA sponges. (11) In regulation of transcription factor localization and sequestration. Adapted with permission from Marchese et al. (2017).

subregions of the V-SVZ sampled from, and therefore under-represent the natural heterogeneities across the niche. Another problem is that equating marker expression with phenotypic state is exceptionally difficult due to the small size and few numbers of certain V-SVZ cells, their dense packing, and fluid gene expression. With the advent of single-cell resolution techniques, more of the molecular mechanisms and gene regulatory networks underpinning these cells are being dissected.

There is another well characterized stem cell niche: the subgranular zone (SGZ) in the hippocampal dentate gyrus (Overall and Kempermann, 2018). Whereas SGZ regulation by lncRNAs is an important topic, in the interest of space, we have mostly focused on the V-SVZ in this paper. However, the same approaches used here to interrogate existing databases of transcriptomic data could also be applied to data from the SGZ.

lncRNAs are regulatory molecules

The non-protein-coding portion of the genome was long dismissed as "junk" DNA, despite making up ~98% of the human genome (Morris and Vogt, 2010). However, it is now known that about 10% of this junk is potentially functionally relevant, and some of it can be transcribed to produce regulatory non-coding RNAs. Long non-coding RNAs (lncRNAs) are a group of molecules afforded rich functional and structural diversity by their ability to associate with both nucleic acids and proteins (Ma et al., 2013). To date, these molecules have been implicated in a vast range of cellular processes, including but not limited to transcriptional regulation, post-transcriptional regulation, and epigenetic control, as summarized in Figure 2. There is a growing body of evidence for the role that some lncRNAs such as *Pnky* and *Paupar* play in the control of neurodevelopmental fate and neurogenesis (Pavlaki et al., 2018; Ramos et al., 2015); a subset of these is thought to work in this context through epigenetic mechanisms and chromatin remodeling, both of which regulate V-SVZ stem cells and neurogenesis. Misregulation of expression of lncRNAs in the brain has been implicated in neurological diseases, from developmental defects to gliomagenesis (Ramos et al., 2016; Zhang et al., 2013).

lncRNAs often show a high degree of cell- and subcellular-specific localization. lncRNA subcellular localization can be regulated via intron retention of nuclear lncRNAs, for example *TUG1* (Dumbovic et al., 2021), or by specific sequences (Lubelsky et al., 2021). They can also be subjected to stronger selection than neutral sequences (Cabili et al., 2015; Derrien et al., 2012), indicative of their functional roles. lncRNAs can be subdivided into five categories based upon their locations relative to protein-coding genes: long intergenic ncRNA, long intronic ncRNA, sense lncRNAs, antisense lncRNAs, and bidirectional lncRNAs (Ma et al., 2013). In addition to genomic classification, other attempts to classify lncRNAs have more recently been made for example based on differences in RNA metabolism (Mukherjee et al., 2017) or in short sequence motif "k-mer" content (Kirk et al., 2018). Each individual lncRNA can

additionally be classed as having *trans*-acting or *cis*-acting mode of action depending on proximity of its interactions.

Some lncRNAs have been observed to accumulate in a localized volume surrounding the domain from which they are expressed, suggesting *cis*-acting mechanisms of action, as is the case for the lncRNA *116HG* (Powell et al., 2013). Such lncRNAs can both activate or repress nearby protein-coding genes using several different mechanisms. For example, *cis*-regulatory regions such as enhancers within the lncRNA locus and/or the process of transcription of the lncRNA can regulate adjacent protein-coding gene expression, rather than the lncRNA gene product itself. Transcriptional interference is thought to be responsible at least in part for the repressive effect of the *Airn*, *Snhg14*, *Kcnq1ot1*, and *Nespas* lncRNAs (Mancini-Dinardo et al., 2006; Meng et al., 2013; Tibbit et al., 2015). Transcriptional interference, however, cannot account for all the repressive effects of lncRNA transcription such as repression of a target gene by a downstream lncRNA or long-range interactions. Following transcription, the lncRNA may remain localized to the domain from which it is expressed and act as a molecular scaffold or guide for the recruitment and direction of epigenetic modifiers (Figure 2) (Pandey et al., 2008; Schertzer et al., 2019; Zhao et al., 2010). Recent work is developing the concept that lowly expressed lncRNAs can accumulate at their sites of synthesis at high concentration and interact with diffusible proteins to form functional territories (Markaki et al., 2021).

Physical interactions between chromatin modifiers and lncRNAs have been demonstrated to be relevant in control of cell identity, as is the case for the CNS-expressed lncRNA *Paupar* (Pavlaki et al., 2018). We found that *Paupar* depletion diminishes OB neurogenesis *in vivo* (Pavlaki et al., 2018; Vance et al., 2014) and that *Paupar*. transcript directly interacts with the KAP1 epigenetic regulator and the transcription factor PAX6, a critical modulator of V-SVZ fate choices This promotes the formation of a chromatin regulatory complex that controls NSC self-renewal and differentiation-relevant gene expression (Ninkovic et al., 2013; Pavlaki et al., 2018; Vance et al., 2014). The lncRNA *Kcnq1ot1* has been shown to bind to two components of PCR2, as well as to the histone methyltransferase G9a (Pandey et al., 2008; Schertzer et al., 2019), mediating the formation of transcriptionally repressed domains. In addition, at some loci, lncRNAs are able to invade the DNA to form an RNA-DNA-DNA triplex in a sequence-specific manner, aiding in the targeting of chromatin remodeling enzymes to specific loci (Mondal et al., 2015).

lncRNAs can form part of a nuclear RNA-RNA interaction network (Chan and Tay, 2018; Imig et al., 2015; Ma et al., 2020; Zhang et al., 2019a). An example of such RNA interactions is *Malat1* binding pre-mRNAs indirectly via protein intermediates (Engreitz et al., 2014). lncRNAs can also act as precursor transcripts for small regulatory ncRNAs, or as molecular sponges for the sequestration of smaller ncRNAs and miRNAs (Figure 2), both of which are the case for *H19* (Kallen et al., 2013; Vennin et al., 2015). Another example describes a network composed of the lncRNA *Cyrano*, two micro RNAs and a circular RNA that collaborate together to regulate neuronal activity (Kleaveland et al., 2018). These mRNA-miRNA-lncRNA interaction networks are thought to be important in maintenance of stem cell identities, with various components consistently differentially expressed between stem cell types (Ma et al., 2020). This is particularly relevant in the context of the V-SVZ, where dysregulation of NSCs can give rise to gliomas.

lncRNAs predominate in brain, especially in human, and are important in brain development and disease

lncRNAs are not only enriched in the brain, but they also show very high cell type and developmental stage specificity that can surpass that of protein-coding genes. The first publication showing lncRNA function in the brain was the Kohtz lab's elegant work on *Evf2* (Feng et al., 2006). This work was in the hippocampus, but *Evf2* is also expressed in the V-SVZ (Figure 3). They demonstrated that the *Evf*2 specifically cooperates with Dlx-2 to increase the transcriptional activity of the Dlx-5/6 enhancer (Feng et al., 2006). Then they showed that *Evf2* binds the DLX and MECP2 transcription factors, regulates expression of *Dlx5/6* and *GAD1*, and is necessary for postnatal hippocampal and dentate gyrus neurogenesis and maintenance of adult hippocampal circuitry (Bond et al., 2009). Recently the Kohtz group has demonstrated that *Evf2* regulates megabase distant gene expression and functionally distinct *cis* versus *trans* effects (Cajigas et al., 2018).

Another seminal paper from the Lim lab discovered that NSCs of the V-SVZ are particularly enriched for a large number of different lncRNAs, relative to surrounding tissues, reporting upwards of 2,000 NSC-unique lncRNAs (Ramos et al., 2013) (Figure 3). For some lncRNAs, there is growing evidence from high-resolution multi-omics studies for important roles, not only in the development of the human nervous system, but also in healthy aging and response to disease. The Lim group went on to show that the lncRNA *Pinky* has different effects at different stages of the lineage, by interacting with a key RNA splicing regulator PTBP1 (Ramos et al., 2015). Together, these studies pointed to a role for regulatory lncRNAs in the maintenance of a healthy, self-renewing NSC population. However, lncRNAs still remain a relatively neglected class of regulatory molecules, and the breadth and depth of their roles in neurodevelopment and neurogenesis have yet to be fully uncovered. There are also many evolutionary questions regarding lncRNAs that remain unanswered.

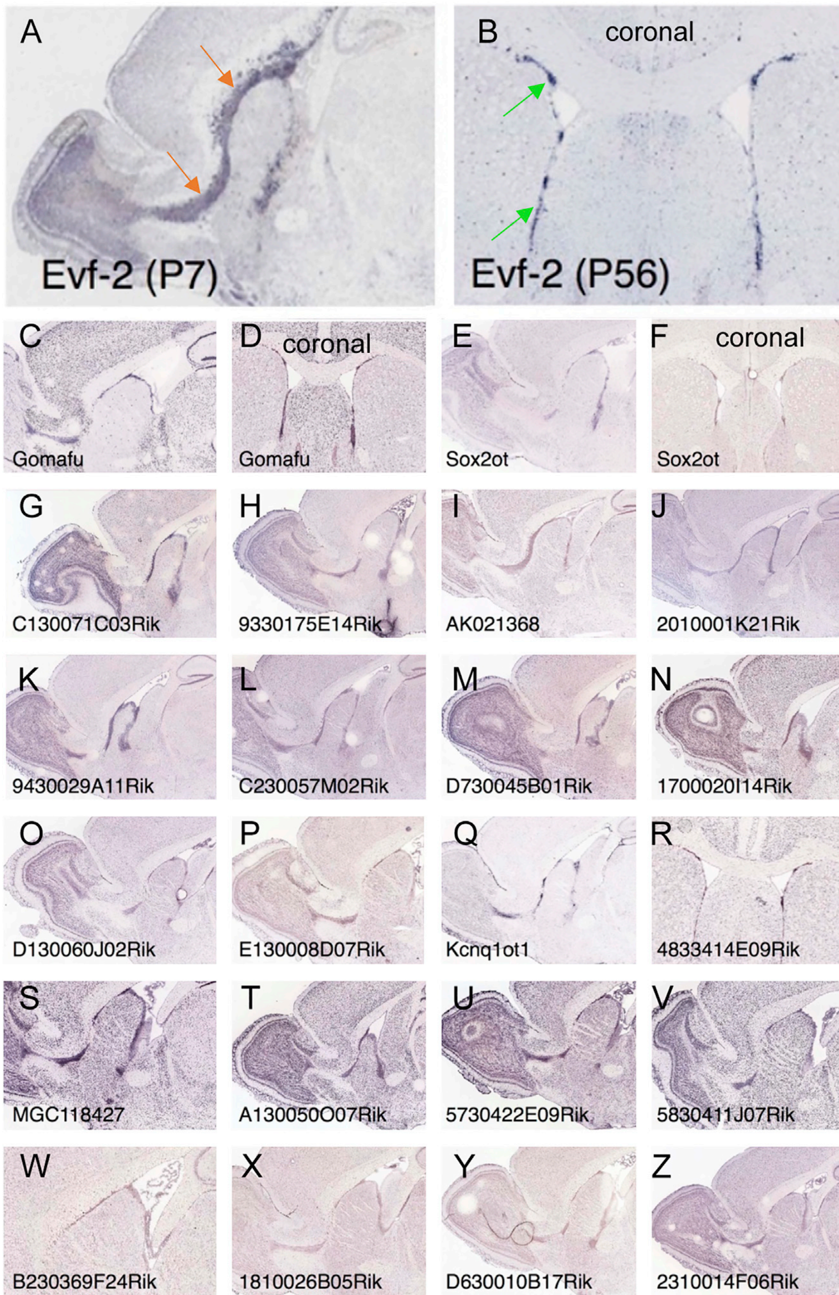

Figure 3. lncRNAs in the V-SVZ, an alphabet of expression

(A–Z) *In situ* hybridizations of lncRNAs in the adult brain. Images taken from the Allen Brain Atlas (Lein et al., 2007). All images are in the sagittal plane, except for (B), (D), and (F), which are coronal. (A, B) Evf-2 expression in the P7 and P56 brain is high in the rostral migratory stream (orange arrows) and in the SVZ (green arrows). Note that some lncRNAs are expressed almost exclusively in the SVZ/RMS (I, Q), whereas others are expressed in the neurogenic niche as well as other brain areas (T, U). Many of the lncRNAs shown are also expressed in the olfactory bulb, the target of the RMS (H, K).

As with mutations in many developmentally expressed genes, the consequences arising from misregulation of lncRNAs can have profound impacts, ranging from developmental defects to tumorigenesis. Tumors, for example gliomas, can be generated from V-SVZ cells, and it is likely that some of these are regulated by lncRNAs. *HOTAIR*, *Hox transcript antisense intergenic RNA*, is a well-studied example of a lncRNA that regulates the V-SVZ and that functions in tumorigenesis (Ramos et al., 2016; Zhang et al., 2015). *HOTAIR* expression is upregulated in glioblastoma multiforme (GBM), and its expression is positively correlated with glioma grade and therefore considered a prognostic factor for GBM patients (Zhang et al., 2013). *HOTAIR* directly interacts with the EZH2 component of the PRC2 complex (Zhang et al., 2015), and knockdown of *HOTAIR* or EZH2 prevents glioma cell proliferation (Zhang et al., 2015). *HOTAIR* knockdown also inhibits GBM cell invasion and migration (Zhang et al., 2015). Future scRNA-seq studies will be essential to show how mutations in lncRNAs regulate gene expression in various V-SVZ cells during tumorigenesis.

In contrast to the damage V-SVZ cells can cause when they become tumorigenic, they are beneficial when they migrate into and help repair brain injury and disease (Chang et al., 2016; Young et al., 2011). Current scRNA-seq papers have laid the groundwork for signaling networks involved in V-SVZ cell homeostatic migration, and future studies will show how they function to regulate emigration and injury repair. Several lncRNAs have already been found to have important functions in CNS disease and are possible biomarkers and therapeutic targets (Fan et al., 2020b; Wei et al., 2018). The lncRNA *H19* is the highest upregulated lncRNA in V-SVZ NSCs in a rat model of stroke (Fan et al., 2020a). It is at least partially necessary for the endogenous increases in proliferation and neurogenesis induced by stroke (Fan et al., 2020a). As discussed below, *H19* regulates small ncRNAs and miRNAs, and it will be important to study how these interactions affect stroke outcomes. In addition to their involvement in stroke, lncRNAs may also regulate V-SVZ neurogenesis in Alzheimer's disease (AD). V-SVZ cell proliferation, migration, and neurogenesis are diminished in mouse models of AD (Esteve et al., 2022; Scopa et al., 2020), and the lncRNA *BACE1-AS* has been implicated in AD (Faghihi et al., 2010). *BACE1-AS* increases BACE1 mRNA stability, Aβ production, and plaque accumulation, worsening disease (Faghihi et al., 2010). AD is characterized by senile plaques, which consist of toxic β-amyloid (Aβ) generated by BACE1 (β-amyloid precursor protein cleaving enzyme). *BACE1-AS* is transcribed antisense to the BACE1 gene and is highly expressed in AD. *BACE1-AS* hybridizes with BACE1 mRNA, blocking miR-485 binding and transcript degradation. We and others have shown that the V-SVZ response to brain injury is quite variable from disease to disease, so it will be important to dissect lncRNA cell-specific effects on disease.

Single-cell RNA sequencing: A brief summary

scRNA-seq was first described in 2009 in a four-cell blastomere (Tang et al., 2009), although the procedure used was highly time consuming and labor intensive (Figure 4). Techniques were developed in 2014 and 2015 to reduce the number of required preparation steps as well as to demonstrate the utilization of microfluidics to isolate single cells and to generate self-contained reactions in individual droplets (Macosko et al., 2015; Patel et al., 2014). The general workflow for cell preparation in scRNA-seq starts with dissecting the tissue of interest; dissociating this tissue to single cells; isolation and barcoding of these cells; reverse transcription to generate cDNAs; library preparation; sequencing; and data analysis. However, there are variations in exactly how each step is performed, each presenting their own strengths and weaknesses. scRNA-seq is a relatively new technique that has gained rapid popularity due to the high resolution of data that it produces. There are many different variations and spin-off techniques that allow integration of different methodologies to provide further information such as spatial resolution (Zhu et al., 2018). While scRNA-seq methodologies confer their own biases and weaknesses, they have revolutionized research into both basic biology and biomedical research by providing insight into local and cell type-specific heterogeneity of transcriptomes, revealing rare or underrepresented cell types that may have been missed by microarray- or bulk-sequencing-based approaches (Nguyen et al., 2018). The resolution and specificity are particularly relevant in an era of progression toward personalized medicine: it has long been acknowledged that within tumors, for example, there is significant cellular heterogeneity that can impact the outcome of treatments. In this context, scRNA-seq can inform the most effective course of treatment given the transcriptomic profile of the tumor.

scRNA-seq allows discovery of gene regulatory networks (GRNs) (Fiers et al., 2018; Iacono et al., 2019). Rather than describing expression and function of one or a few genes at a time, it shows dynamic progression of the ensemble of transcripts (Street et al., 2018). In addition to simple transcriptomic readouts, scRNA-seq can help reveal gene fusions, splicing variants, and mutations (Li and Wang, 2021). Many scRNA-seq papers in the V-SVZ have gone beyond simple transcriptomics analysis of cells in homeostasis and have probed transcriptomic changes in development, aging, inflammation, or brain injury (Bitar et al., 2022; Borrett et al., 2020; Dulken et al., 2019; Kalamakis et al., 2019). This approach can be vital for understanding dynamic gene expression changes in the course of disease. Not only can scRNA-seq map cell subtype-specific changes in GRNs over time, but it can also help drug discovery by mapping how therapeutic interventions enable reversal of pathogenic GRNs (Kim et al., 2020).

There are several challenges in scRNA-seq (Lahnemann et al., 2020), but perhaps the greatest for the V-SVZ is loss of spatial resolution in the data (Lee et al., 2022). This can be in part overcome using a combination of known gene markers for specific anatomical locations, although this is dependent on *a priori* knowledge of these markers and may have limited anatomical resolution. This is particularly relevant in the context of scRNA-seq in the V-SVZ as samples are frequently contaminated with surrounding tissue, for example from the striatum or choroid plexus (Olney et al., 2022). There are many emerging variations of scRNA-seq that consider spatial location of cells, such as "cell2location" that allows spatial information to be integrated into the data analysis (Kleshchevnikov et al., 2022). Another potential problem can be transcriptomic programs that are activated upon dissociation: cells can respond to changes that occur during early sample preparation, which may confound downstream analyses. Macrophages and microglia are notorious for changing their

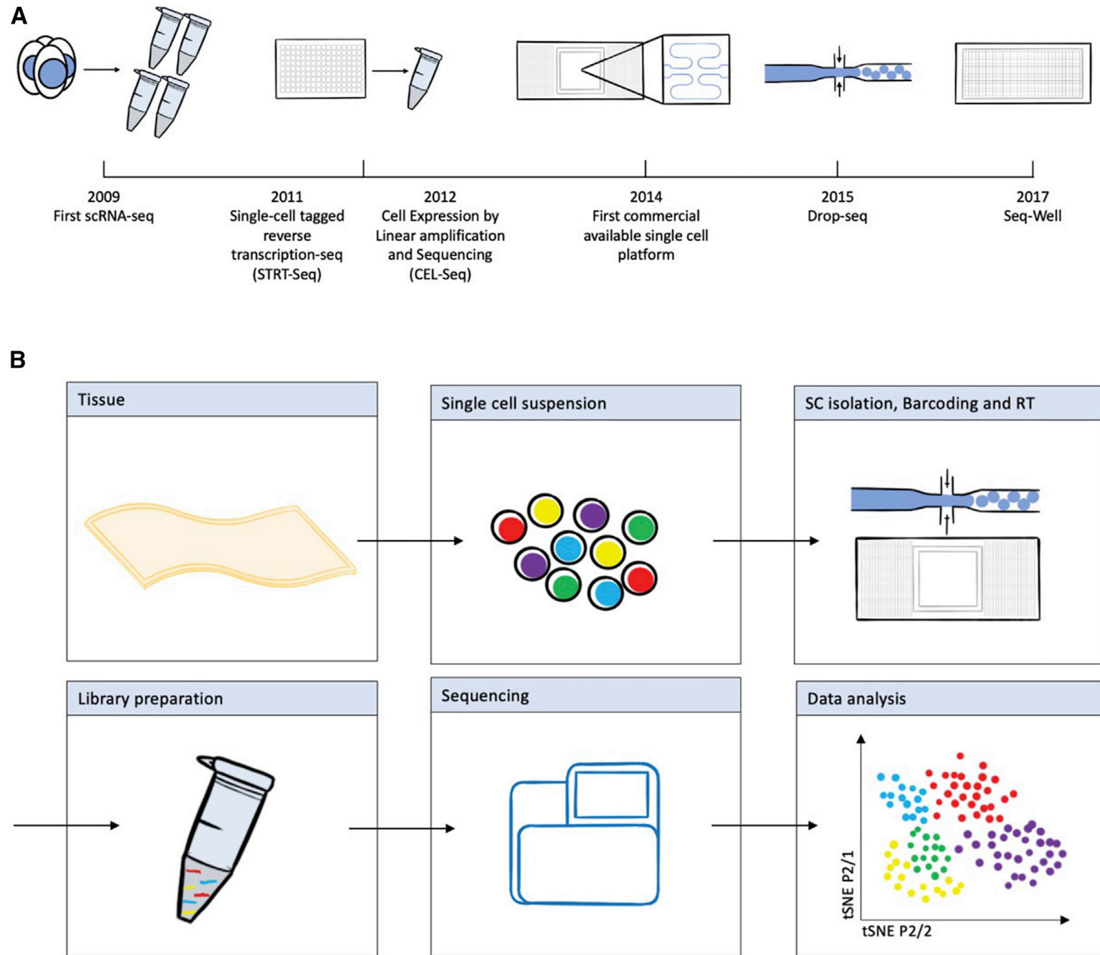

Figure 4. Overview of single-cell sequencing

(A) Timeline of single-cell RNA-seq technical development from first description by Tang et al. (2009) to the development of seq-well by Gierahn et al. (2017).

(B) Process of single-cell RNA-seq from tissue isolation to data analysis. Adapted from Wu et al. (2018) with permission from Elsevier.

transcriptomes rapidly upon removal from brain (Gosselin et al., 2017) and in response to various pathologies (Masuda et al., 2020).

An important question relates to the relatively poor sensitivity of scRNA-seq in detecting low-abundance transcripts such as lncRNAs, and it is likely that there are false negatives in the literature (Lahnemann et al., 2020; Vieth et al., 2019). Targeted scRNA-seq or bulk-sequencing approaches such as Probe-seq that pre-selects cell subtypes might be helpful in detecting low-abundance lncRNAs (Amamoto et al., 2019). However, these will be more focused studies, rather than unbiased screens. A central decision must be made at the start of scRNA-seq-based study regarding depth of reads versus numbers of cells (Lahnemann et al., 2020; Vieth et al., 2019). As well, there are important differences between cell isolation and RNA extraction procedures that must be considered to ensure comparability to other studies (Lahnemann et al., 2020; Vieth et al., 2019). Finally, amplification biases have strong dependence on PCR-based methods, make comparing expression of different genes difficult (Lahnemann et al., 2020; Vieth et al., 2019).

There are many approaches to bioinformatically interrogate lncRNA datasets (Vieth et al., 2019). As an example, we typically carry out the following sequence of data curation and analysis to identify cell type-specific roles for lncRNAs and protein-coding genes in the V-SVZ using loss-of-function models. Different cell subtypes in the V-SVZ are classified based on expression signatures and developmental trajectories of V-SVZ lineage progression reconstructed at single-cell resolution. Data are first assessed and filtered through the Cell Ranger platform and then analyzed within Seurat enabling sample quality control, identification of outlier cells, correction of technical biases,

normalization, feature selection, and clustering. To capture differences in cell trajectories between conditions, we compare pseudo-time trajectories and identify genes differentially expressed between conditions and along the pseudo-time trajectory. We explicitly test for differences along pseudo-time. We perform trajectory inference on all cells across conditions at once using Monocle3 or SlingShot to identify the most stable trajectory. Within-lineage differences in distributions of pseudo-time values between conditions are assessed using Kolmogorov-Smirnov tests followed by false discovery rate analysis. We apply SCENIC to reconstruct a cell type-specific regulatory network, enabling us to highlight the impact of perturbations. This fully integrated analysis makes full use of the data we generate.

RESULTS

Integrated analysis of scRNA-seq datasets in the V-SVZ

General conclusions about the V-SVZ

scRNA-seq studies have provided great insight into the function of the V-SVZ and have revealed many nuances of NSC subtypes. An important factor highlighted by scRNA-seq has been a previously underappreciated heterogeneity within individual NSC clusters (Llorens-Bobadilla et al., 2015). Bulk RNA sequencing studies allowed the separation of NSCs into activated (aNSC) and quiescent (qNSC) subpopulations. Whereas, scRNA-seq has allowed the resolution of aNSCs and qNSCs into multiple transcriptionally distinct phases, defined on a study-by-study basis depending on the expression of select genes (e.g., Olig2) (Del Águila et al., 2022) or their position along a spectrum from quiescent to activated (Kalamakis et al., 2019). While the delineations between these subtypes vary between individual studies, the overall conclusion remains that NSCs are much more complex and variable than simply "activated" or "quiescent." Single-cell studies have also pointed toward function; they identified a sub-population of cells that respond to injury (Llorens-Bobadilla et al., 2015), have shown where gliogenesis occurs in the niche (Delgado et al., 2021), and have even identified genes that could act as drug targets in V-SVZ-derived cancers (Wang and He, 2019).

General conclusions about lncRNAs in the V-SVZ

We started by examining data to identify lncRNAs that were enriched in NSCs relative to other cell types within the V-SVZ niche (Table S1, Spreadsheet S1). A comparison could be made following dimensionality reduction and cell type clustering in the scRNA-seq pipeline, followed by comparing average gene expression within a given cluster relative to other clusters. This can be performed by the Seurat "FindAllMarkers" function, among other approaches (Hao et al., 2021) (Table S1). Examination of the output from this analysis in multiple studies revealed consistent results from a disparate range of approaches, as described below. As discussed, differences in methodology can impact the overall outcome of the analysis; however, the findings by independent studies with independent methodologies of the same results reinforce the strength of individual claims. Nevertheless, consistent patterns of cell type enrichment, while informative, do not confer or confirm function.

We investigated the results of eight papers (Table 1) that performed an analysis of cell type enrichment within the V-SVZ niche in healthy control adult mice (del Águila et al., 2022; Cebrian-Silla et al., 2021; Kriska et al., 2021; Llorens-Bobadilla et al., 2015; Mizrak et al., 2019; Mizrak et al., 2020; Zamboni et al., 2020; Zywitza et al., 2018). Each time a particular lncRNA was found to be enriched in an NSC cluster ($p < 0.05$), we assigned a score of 1. Across all eight datasets, we summed the scores for each individual lncRNA, giving a total score for ("cumulative occurrences," CO) (Table S1). We performed this calculation for NSCs, transit amplifying progenitors, and neuroblasts.. lncRNAs that showed enrichment in one of these cell types in half or more of the datasets examined (≥ 4) are shown in supplementary tables and supplementary spreadsheets. Subdividing these data to examine lncRNAs that are selectively enriched in aNSCs (Table S3, Spreadsheet S2) reveals many novel lncRNAs, as well as some with described functions such as *Erdr1* (eryhthroid differentiation regulator 1), *Lockd* (lncRNA downstream of Cdkn1b), *Miat* (myocardial infarction associated transcript), *Pantr1* (POU3F3 adjacent non-coding transcript 1), *snhg14* (small nucleolar RNA host gene 14), *sox2ot* (SOX2 overlapping transcript), and *Airn* (antisense of IGF2R non-protein-coding RNA). As might be anticipated, many of these lncRNAs have been found to play a role in regulation of cancers (Table S3).

As discussed, the neurogenic capacity of the V-SVZ declines with age, and this is likely not exclusively due to stem cell depletion (Kalamakis et al., 2019). Our literature search identified three papers examining the effect of age on the murine V-SVZ, two of which had the necessary supplementary data available online. We examined these studies (Kalamakis et al., 2019; Xie et al., 2020) looking at the aging stem cell niche, with a focus on lncRNAs whose expression increases in quiescent NSCs with age, with their accumulation perhaps regulating activation (Table S2, Spreadsheets S3, S4,S5). Across these two papers, there were 14 lncRNAs that were found in both studies to be upregulated with age in qNSCs.

Notably, many of these lncRNAs are novel, with no functional annotation or documentation beyond the supplementary tables of scRNA-seq papers. As there are limited functional annotations available for these genes, gene

Table 1. Comparison of parameters in papers on scRNA-seq in the V-SVZ

Paper	Year	Age	Sex	Strain background	Region of SVZ	Average number of genes covered per cell	Number of cells profiled	Average reads per cell	Sequencing method	scRNA-seq platform/ library generation
Llorens-Bobadilla et al.	2015	8–12 weeks	male	C57BL/6	lateral wall	not explicitly reported	337 per repeat	5,783,221.4 mapped reads average and 4,998,769.7	Illumina HiSeq 2000	Smart-seq2
Shi, Z. et al.	2018	29 and 2 months	not reported	C57BL/6	lateral wall, whole wall	∼3,000 genes per cell	1,465 cells	4,611,392.5 mapped reads mean	Illumina deep sequencing	Smart-seq2
Zywitza, V. et al.	2018	2–4 months	male and female	C57BL6	whole SVZ	median 734 genes and 1,137 UMIs per cell	9,804 cells	not explicitly reported	Illumina NextSeq 500	Drop-seq
Basak, O. et al.	2018	Not reported	not reported	C57BL/6	medial and dorsal walls	23,480 gene loci	1,465 cells	not explicitly reported	Illumina HighSeq 2500	CEL-seq
Kalamakis, G. et al.	2019	2, 7, 8, and 22 months	male	C57BL/6	not explicitly reported	median genes per cell 2,010, 2,355	1,849 (old mice), and 2,088 (young mice)	193,606 and 172,528	Illumina Novaseq 6000	Smart-seq2, 10X Genomics Chromium
Mizrak, D. et al.	2019	8–10 weeks	male and female	C57BL/6J	lateral and septal, separated in analysis	not explicitly reported	not reported	not explicitly reported	Illumina Novaseq6000	Drop-seq
Mizrak, D. et al.	2020	P56 and P70	male and female	C57BL/6J	lateral and septal walls	not explicitly reported	>56,000 cells from matched V-SVZs and OB. 12,334 and 7,903 from NESFPLO and GCERT2 datasets	not explicitly reported	Illumina NextSeq 500	Drop-seq
Xie, X. P. et al.	2020	2 weeks; 2, 6, and 12 months	not reported	C57BL/6	whole SVZ	not explicitly reported	5,600 cells	not explicitly reported	Illumina	Drop-seq
Zamboni, M. et al.	2020	>2 months	female	C57BL/6j	not explicitly reported	1,986 genes/ cell on average	5,997 cells	not explicitly reported	Illumina NextSeq 500	10x Genomics Chromium

(*Continued on next page*)

Table 1. *Continued*

Paper	Year	Age	Sex	Strain background	Region of SVZ	Average number of genes covered per cell	Number of cells profiled	Average reads per cell	Sequencing method	scRNA-seq platform/ library generation
Cebrian-Silla, A. et al.	2021	P29 and P35	male and female	CD1-elite/J	lateral wall	not explicitly reported	∼31,000	total of 2,892,555,503 reads for all cells	Illumina NextSeq 500	10x Genomics Chromium
Kriska, J. et al.	2021	P50–56	male	C57BL/6	lateral wall	not explicitly reported	not reported	not explicitly reported	Illumina NextSeq 500	10x Genomics Chromium
Chen, X et al.	2021	8–10 weeks	male	C57BL/6J	lateral wall	not explicitly reported	15,000	not explicitly reported	Illumina deep sequencing	10X Genomics Chromium
Belenguer, G. et al.	2021	Not reported	not reported	C57BI6/J	whole SVZ	∼16,000 protein-coding genes per sample	200 to 10,000 cells (n = 3–4 independent samples per population)	average of 15 million 75nt single-end reads	Illumina NextSeq 500	Not explicitly reported
Del Aguila, A et al.	2022	P14 and P16, 10–12 weeks	male and female	C57BL/6J	not explicitly reported	median of ∼3,000 genes/cell	17,941	normalized to 10,000 molecules per cell	Illumina NextSeq 500	10x Genomics Chromium

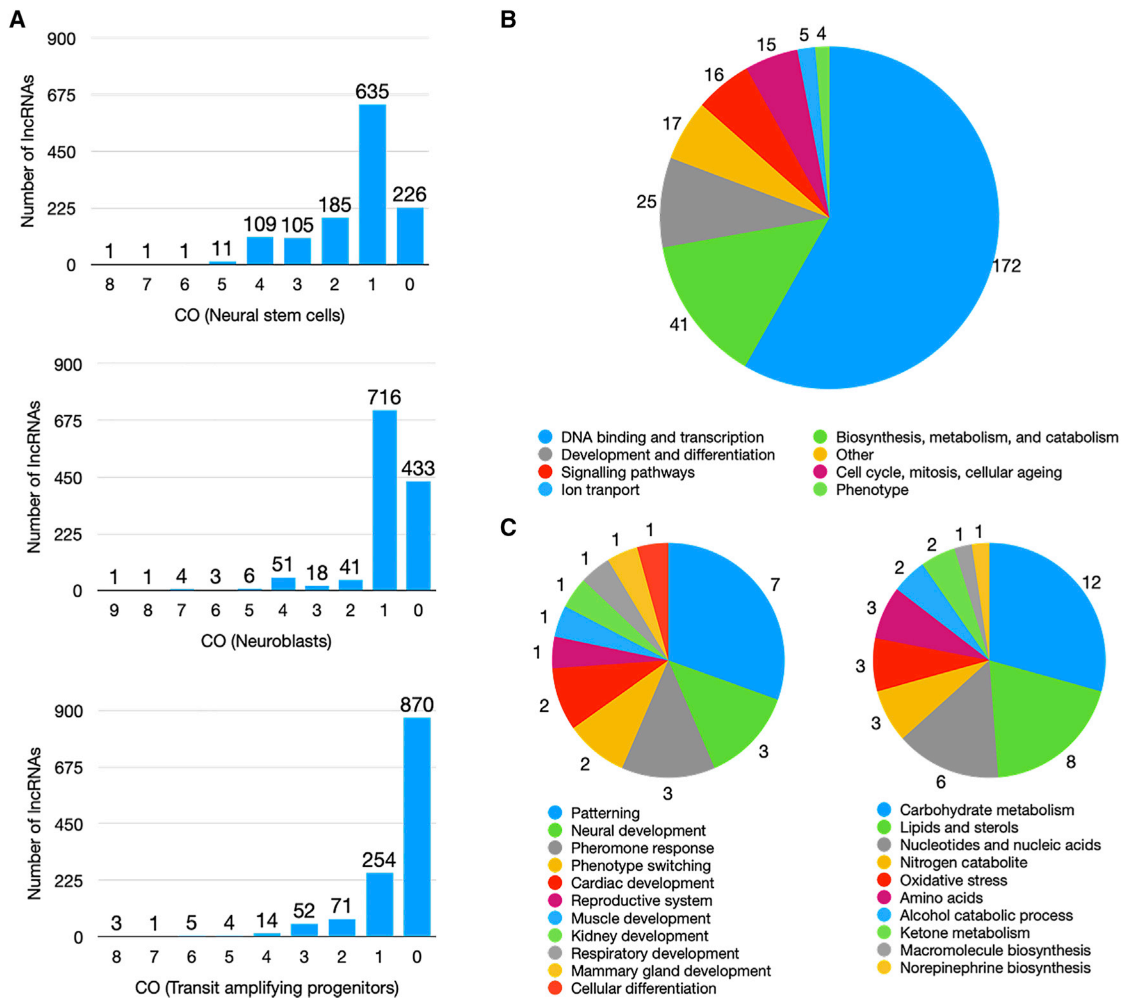

Figure 5. Data summary for scRNA-seq analysis

(A) Results of aggregation of lncRNA enrichment data plots showing the distribution of lncRNA genes with each cumulative occurrence (CO) value, divided into NSCs (top), neuroblasts (middle), and TAPs (bottom). As shown, the majority of lncRNAs have a low value of CO (0–3), indicating that their enrichment in a given cell type is not replicated between studies. A small number of lncRNAs are shown to be enriched in the selected cell type in many different studies, indicated by a higher value for CO.

(B) Examination of the ontologies of transcription factors that bind the promoters of lncRNAs that accumulate in qNSCs with age. Ontologies that were significantly associated ($p > 0.05$) with every lncRNA promoter examined were categorized into eight groups: DNA binding and interaction, development and differentiation, signaling pathways, ion transport, biosynthesis metabolism and catabolism, cell cycle mitosis and cellular aging, disease phenotypes, and "other." The chart represents the proportion of these GO terms that fell into each category.

(C) Breakdown of GO terms in the two largest categories after DNA binding: "Development and differentiation" (left), and "Biosynthesis, metabolism, and cellular aging."

ontology (GO) analysis could not be performed. Therefore we instead identified transcription factors with enriched binding motifs in the promoters of each gene via PSCAN, and ran GO on these sets of genes. Examination of GO terms that were associated (EnrichR padj < 0.05) with the cohort of transcription factors that bind promoters of the 14 lncRNAs that accumulate in qNSCs with age reveals interesting trends (Spreadsheets S4, S5). Among the 296 GO terms that were associated with all lncRNA promoters examined, 41 are associated with transcriptional regulation of and response to metabolism and metabolites (Figure 5, Spreadsheet S5). Additionally, there are many terms that are known to be important in the self-renewal and aging of stem cells such as bone morphogenetic protein (BMP) signaling, telomere extension, and lipid metabolism (Ferrón et al., 2009; Lim et al., 2000; Sênos Demarco et al.,

2020) (Spreadsheet S6 "GO up with age"). Moreover, there are several terms associated with regulation of mitosis and exit from the cell cycle (Spreadsheet S6 "GO up with age").

Among these 14 lncRNAs, whose expression increases in qNSCs with age, only one has been extensively studied: *Kcnq1ot1* (Kcnq1 opposite strand/antisense transcript 1), a paternally expressed lncRNA. *Kcnq1ot1* is encoded on the opposite strand to Kcnq1, and it is part of a primary imprinting control region (Mancini-DiNardo et al., 2006). Mutation, abnormal methylation, or uniparental disomy at this locus can be causative of Beckwith-Wiedemann syndrome, an overgrowth syndrome with an increased predisposition to cancers (Lee et al., 1999; Smilinich et al., 1999; Waziri et al., 1983). Furthermore, *Kcnq1ot1* has been found to have multiple effects linked to brain injury and neuroinflammation. Its knockdown alleviates neuronal deficits following traumatic brain injury (Liu et al., 2021), and its expression exacerbates reperfusion injury (Yi et al., 2020).

The initial calculation of CO was then supplemented by examination of *in situ* hybridization (ISH) data, conservation, and genomic context, as well as interrogation of the literature of a select set of lncRNAs to validate their potential for NSC regulation in the V-SVZ. Examination of ISH data available from the Allen Brain Atlas revealed specific lncRNA localization to the V-SVZ, frequently to the rostral migratory stem and occasionally to the OBs (Sunkin et al., 2013). These lncRNAs were frequently expressed in the SGZ of the DG, indicating enrichment in the two NSC niches of the mammalian brain. This is exemplified by lncRNAs such as *Sox2ot* and *Evf2* (Figure 3).

Sox2ot

Sox2ot was found in independent studies to be enriched in multiple cell types along the neurogenic lineage and to be increased in expression with NSC activation (Mizrak et al., 2020; Mizrak et al., 2019). ISH data from the Allen Brain Atlas shows that it is localized to the murine neurogenic niches as well as being enriched in the OB in a layer-specific manner (Figure 6). We found that *Sox2ot* is conserved between humans and mice, and both the human and mouse gene promoters are enriched for transcription factor binding motifs associated with NSCs and neural crest differentiation (Figure 6, Spreadsheet S6), suggesting functional relevance of this locus in V-SVZ NSCs (PSCAN $p < 0.05$, EnrichR padj < 0.05). This inference is further reinforced by bulk RNA-seq data from a murine model showing that *Sox2ot* is significantly upregulated during gliomagenesis from the V-SVZ, indicating that it is perhaps participating in a mechanism to increase stem cell activation and proliferation (Bardella et al., 2016). A literature search validates this prediction by revealing functional studies showing the role of *Sox2ot* in neural development, neurogenesis, and gliomagenesis (Amaral et al., 2009; Shahryari et al., 2015). It is thought to operate in multiple ways to modulate neurogenesis, such as through regulation of the *Sox2* enhancer or via miRNA sponging (Amaral et al., 2009; Lin et al., 2020).

Xist

We found that expression of the lncRNA *Xist* (*X-inactive specific transcript*) increases with age and with stem cell activation (n = 3 studies aging, n = 2 activation). The canonical role of *Xist* is in X chromosome silencing during early development in females (Brown et al., 1991), but *Xist* has broad ranging roles in development and regulation of inflammation (Sun et al., 2018; Wang et al., 2021; Zhang et al., 2019b). *Xist* drives inflammation in multiple contexts; its depletion can reduce the expression of inflammatory markers, and upregulation increases inflammation and can even lead to amyloid Aβ accumulation (Yan et al., 2022b). Inflammation is a key factor that regulates neurogenesis in the V-SVZ, and inflammatory markers increase with aging in the V-SVZ (Bardella et al., 2018). While the role of *Xist* in this context has yet to be elucidated, it is likely that it is important for regulating neurogenesis via modulation of inflammation and inflammatory responses in the niche. Furthermore, two more lncRNAs involved in X chromosome silencing (*Tsix*, *Jpx*) were found to be differentially expressed with stem cell aging, although this trend has been recapitulated in fewer studies than Xist (Table S1).

Miat

Miat (*myocardial infarction associated transcript*) (aka Gomafu) was found to be increased in expression with stem cell activation in three independent datasets, and ISH data show localization to the V-SVZ and SGZ (Figure 3) (Sunkin et al., 2013). While *Miat* function has not been studied in the context of V-SVZ neurogenesis, examination of binding site motifs in the mouse and human *Miat* promoters via PSCAN and EnrichR reveals that this locus is associated with neurodegenerative disease such as Alzheimer's disease, Huntington's disease, and Friedrich's ataxia (Spreadsheet S7). This is consistent with a documented role of this lncRNA in suppression of neuronal apoptosis (Li et al., 2019). *Miat* has also been proposed as an important modulator of the response to ischemic injury (Guo et al., 2021; Li et al., 2019; Zhang et al., 2021). These data suggest that *Miat*'s functions in the V-SVZ are primarily linked to injury response.

Imprinted lncRNAs: (Meg3, Kcnq1ot1, Snhg14)

We found three imprinted lncRNAs, *Meg3*, *Kcnq1ot1*, and *Snhg14*, in our analysis of V-SVZ lncRNAs. Given the number of annotated lncRNAs in the mouse genome (10,206) (Howe et al., 2021) and the number of imprinted lncRNAs (estimated ~76) (Santini et al., 2021), this is a high enrichment of imprinted lncRNAs. Genomic imprinting is an epigenetic process by which a gene is expressed monoallelically depending on the parent from which the allele was inherited. Imprinted genes play diverse roles in development

A

B

Normalised expression

0 1 2 3 4 5

Cell type

Inhibitory interneurons
Ependymal cell
Radial glia
Oligodendrocyte progenitors
Neural tube
Schwann cell precursor
Neural progenitor cell
Isthmic organiser cells
Inhibitory neurons
Lens

C

Neural Crest Differentiation WP2074
Nuclear Receptors WP509
PluriNetWork WP1763
IL-3 Signaling Pathway WP373
Dopaminergic Neurogenesis WP1498
Retinol metabolism WP1259
Heart Development WP2067
Endochondral Ossification WP1270
Wnt Signaling Pathway and Pluripotency WP723
Ethanol metabolism resulting in production of ROS ...

Gene count

P value
0.005
0.010
0.015

Neural Crest Differentiation WP2084
Dopaminergic Neurogenesis WP2855
Nuclear receptors WP170
Endoderm differentiation WP2853
Cell Differentiation - Index expanded WP2023
PPAR-alpha pathway WP2878
Adipogenesis WP236
Energy Metabolism WP1541
Sudden Infant Death Syndrome (SIDS) Susceptibility...
Pre-implantation embryo WP3527

Gene count

P value
0.004
0.008
0.012

D

Eldr
Eldr
Gas5
Translation
Snhg14
Sox2ot
Gas5
Airn
accumulation of Evf2 lncRNA
Rian
Evf2
Miat
Meg3
Kcnq1ot1
Nucleus
Cytoplasm

lncRNA
miRNA
G9a
ILF3
PRC2
DNmt3a
GR

Figure 6. Sox2ot case study

(A) Expression of Sox2ot in mouse brain, determined by ISH. Data from Allen Brain Atlas.

(B) Expression of Sox2ot in different cell types. Data from Mouse Organogenesis Cell Atlas.

(C) EnrichR output for WikiPathways 2019 Mouse (left) and WikiPathways 2021 Human (right), for genes with overrepresented binding sites in the mouse or human Sox2ot promoters. Binding site identified by PSCAN $p < 0.05$.

(D) Selected roles of 10 V-SVZ NSC-enriched lncRNAs. These are not necessarily the only roles of these lncRNAs or the exact mechanism of their action within NSCs. (Clockwise) *Eldr* (CO: 4 NSC, 5 TAP), *Gas5* (CO: 4 NSC, 5 NB, 6 TAP), *Airn* (CO: 3 NSC, 3 aNSC), *Evf2/Dlx6os1* (CO: 6 NB, 4 TAP), *Kcnq1ot1* (CO: 3 Neuroblasts, 2 qNSC), *Meg3* (CO: 9 NB), *Miat* (CO: 5 NSC, 7 NB, 4 TAP), *Rian/Meg8* (CO: 4 NB), *Sox2ot* (CO: 5 NSC, 7 NB, 4 TAP), *Snhg14* (CO: 4 NSC, 5 TAP).

and metabolism, and mutations in these genes or the loci that control their expression frequently give rise to syndromes characterized by neurodevelopmental defects (Mackay and Temple, 2017). Of note in this data are the paternally expressed *Kcnq1ot1* and *Snhg14*, as well as the maternally expressed *Meg3*. *Meg3* has a characterized role in many types of cancer where it acts as a tumor suppressor across a broad range of hypothesized mechanisms, including via regulation of p53 and TGF-beta (Mondal et al., 2015; Tang et al., 2016). Furthermore, *Meg3* can regulate cell death in response to hypoxia/ischemia after stroke (Yan et al., 2016). In health, *Meg3* is mono-allelically expressed at high levels in the dentate gyrus of the hippocampus where is it able to regulate SGZ neurogenesis, as well as in the OB (McLaughlin et al., 2006), the target of V-SVZ neurogenesis (Altman, 1969). We found that the paternally expressed *Kcnq1ot1* was increased with stem cell activation, increased in expression with age, and showed cell type specificity of expression. The precise role of *Kcnq1ot1* in healthy neurogenesis has yet to be elucidated, although given our results and its role in cancer (Weksberg et al., 2001), it is likely to be important. We also found that the paternally expressed *Snhg14* was differentially expressed with stem cell activation and also increased with age in NSCs. *Snhg14* has been implicated in the response to ischemic damage in the brain following stroke, and it is thought to promote inflammatory responses via microglia activation and miRNA regulation (Qi et al., 2017; Zhong et al., 2019).

lncRNA functions in the SVZ

In Figure 6D we illustrate several of the validated or purported functions of lncRNAs that may be found in the V-SVZ. *Eldr (EGFR long non-coding downstream RNA)* can be found in both the nucleus and cytoplasm and acts via multiple mechanisms. Cytoplasmic *Eldr* transcripts can interact with and sequester Ilf3, allowing cytoplasmic accumulation. It can also interact with and inhibit miRNAs such as miR-7 (Sur and Ray, 2022; Sur et al., 2020). *Gas5 (growth arrest specific 5)* is able to act as a decoy for the glucocorticoid receptor, limiting its binding to DNA glucocorticoid response elements (Kino et al., 2010). Additionally, it can bind eukaryotic translation initiation factor-4E (eIF4E) in the cytoplasm, thus limiting translation of genes such as c-Myc (Hu et al., 2014). *Airn (antisense of IGF2R non-protein-coding RNA)* regulates local gene expression through a combination of transcriptional interference to silence Igf2r, as well as recruitment of the histone methyltransferase G9a to silence other genes such as Slc22a3 (Latos et al., 2012; Nagano et al., 2008). *Evf2/Dlx6os1 (distal-less homeobox 6, opposite strand 1)* accumulates in a local "cloud" encompassing the domain from which it is transcribed. Within this cloud the nearby Dlx5/6 genes are regulated via *Evf2*-dependent recruitment of Dlx2 and MeCP2 (Berghoff et al., 2013; Bond et al., 2009). *Kcnq1ot1 (KCNQ1 opposite strand/antisense transcript 1)* is expressed from the imprinted Kcnq1 domain and interacts with histone methyltransferase G9a and PRC2 components Ezh2 and Suz12 in a tissue-specific manner to regulate local chromatin remodeling (Pandey et al., 2008). *Meg3 (maternally expressed 3)* is able to form RNA-DNA triplexes at enhancer sequences and recruit PRC2 to specifically silence the expression of genes in the TGF-beta pathway (Mondal et al., 2015). *Miat (myocardial infarction associated transcript)* acts as a competing endogenous RNA to bind miRNAs such as miR-150-5p (Sun et al., 2018). Additionally, it can interact with miR-34a in association with the RISC complex and epigenetically silence its expression (Fu et al., 2018). *Rian (RNA imprinted and accumulated in nucleus)* suppresses the expression of miR-34a and miR-203 by specific recruitment of PRC2 to their promoters, and it cooperates with *Meg3* to regulate the TGF-beta pathway (Terashima et al., 2018). *Sox2ot (Sox2 overlapping transcript)* binds the RISC complex component Ago2 as well as miR-200s to regulate the stability of Sox2 mRNA (Li et al., 2018). *Sox2ot* can also interact with YY1 in the nucleus to mediate its interaction with CpG islands in the Sox2 locus (Knauss et al., 2018). *Snhg14 (small nucleolar RNA host gene 14)* has multiple roles in inflammation and cancer, one of which is mediated via a positive feedback loop in which *Snhg14* sponges the miRNA miR-5590-3p (Figure 6 D) (Zhao et al., 2019).

DISCUSSION

Here we have highlighted how a qualitative analysis of multiple scRNA-seq studies can rapidly provide a wealth of information regarding the behavior and expression of lncRNAs within the V-SVZ. We examined this output in conjunction with other open access data sources and analysis platforms to allow inference of genes that are relevant for stem cell activation and regulation without a dependence on high-performance computing or programming expertise. Designing and conducting screens to identify genes that may be of functional relevance is laborious and time consuming. However, in a new age of open access "big data," we are in a prime position to interrogate open access data in a rapid and multi-modal manner.

A general issue with this approach is the ability to directly compare datasets: it is rare that two studies have made use of the same mouse strain, at the same age, and the same sex under identical conditions, and then utilize the exact same methodology to prepare their samples. As such, implementation of a quantitative meta-analysis may not be possible for the NSCs of the V-SVZ currently, due to the small number of studies that are both available and directly

comparable. This is limited even more so by the characteristics of lncRNAs themselves; while they are frequently expressed with a higher cell type and developmental stage specificity than protein-coding genes, they are often very lowly expressed (Derrien et al., 2012). This means that they may be missed when preparing scRNA-seq libraries, so only highly expressed and highly variable lncRNAs are revealed in these data. While this provides "low hanging fruit" in terms of identifying potential molecular regulators, there is still a long way to go in terms of the sensitivity of scRNA-seq technologies.

Additionally, while we can confirm the conservation of lncRNA genes in terms of synteny or sequence conservation, there is still a relative paucity of human or even primate data regarding the V-SVZ and patterns of gene expression in healthy aging brains. Moreover, many of the conclusions that are drawn from single-cell resolution transcriptomic data in healthy tissues are inferential and descriptive, rather than mechanistic.

Many studies regarding the role of specific lncRNAs in the regulation of stem cell activation and proliferation have been conducted in the context of cancer (Bhan et al., 2017). While potentially informative regarding their roles in cancer, these studies neglect the function of these molecules in healthy cells. Examining the expression of lncRNAs under various pathological conditions, however, does reveal roles that we would anticipate to be similar in homeostatic V-SVZ neurogenesis. Gliomas are a prime example of misregulation of the endogenous processes of NSC activation and maintenance within the V-SVZ (Bardella et al., 2016). Therefore, glioma-centric lncRNA studies can theoretically inform V-SVZ functions of lncRNAs, and conversely studies of the healthy V-SVZ can in turn inform glioma studies. Several of the lncRNAs we focus on here are implicated in cancer, including *Sox2ot*, *Kcng1ot1*, *Meg3*, and *XIST*. While their functional roles in other cancers have been studied, their specific function in V-SVZ gliomas are unclear. We generated a gliomagenic phenotype by knocking in *IDH1*^{R132H} into the V-SVZ and found that *Sox2ot* expression was significantly increased (Bardella et al., 2016). Future work will hopefully uncover how *Sox2ot* and these other lncRNAs regulate V-SVZ gliomagenesis.

One of the central problems in the field is regarding the mechanisms that regulate NSC quiescence versus activation. This question intersects with the notion that NSC can be depleted with age via a lifetime of homeostatic or injury-induced activation. There are multiple mechanisms and pathways that contribute to the reduced activation of NSCs with age but then change in function over time. It is important to consider in the case of *Xist* that regulation of stem cells is not its only role: it regulates inflammation and inflammatory responses. The impact of *Xist* expression on inflammation appears to be context dependent and has not yet been investigated in the context of NSCs. Some studies have found that it promotes inflammation (Chen et al., 2021; Yan et al., 2022a) and others that it reduces inflammation (Shenoda et al., 2021; Wang and Cao, 2022). Therefore the apparently contradictory patterns of expression observed in *Sox2ot* and *Xist* may be due to multiple regulatory mechanisms acting in parallel. It is currently unclear whether these observed patterns of expression change are mechanistically or functionally relevant as the action of *Sox2ot* and *Xist* have yet to be investigated in the context of the aging V-SVZ.

In conclusion, the approaches presented in this paper curate a pool of lncRNA genes that may have functional relevance in the V-SVZ, providing utility both through extending our understanding of lncRNAs in this niche but also in informing future therapeutic approaches. RNA-mediated therapeutics are coming of age (Winkle et al., 2021), and the high specificity of lncRNA expression makes them prime targets as side effects may be significantly less than with current drugs. This is of relevance in the context of stroke, Alzheimer's disease, and other neurodegenerative disease where lncRNA-modulating therapeutics could stimulate neurogenesis to ameliorate damage .

EXPERIMENTAL PROCEDURES

Resource availability

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Materials availability

This work did not generate any unique materials.

Data and code availability

https://github.com/JemimaBecker/Single-cell-sequencing-in-the-V-SVZ.

Qualitative meta-analysis

A literature search was conducted to identify research articles that made use of single-cell RNA sequencing in the V-SVZ using the following searches in Scopus: (TITLE-ABS-KEY ("V-SVZ" OR svz OR sez OR "subventricular zone" OR "subependymal zone") AND TITLE-ABS-KEY ("scRNA-seq" OR "single-cell sequencing")) and in GEO DataSets: ((V-SVZ OR SVZ OR subventricular OR subependymal OR SEZ)) AND "mus musculus"(Organism). The majority of papers identified document the murine V-SVZ, and 14 of these were chosen for analysis (Basak et al., 2018; Belenguer et al., 2021; Cebrian-Silla et al., 2021; Del Aguila et al., 2022; Kalamakis et al., 2019; Kriska et al., 2021; Llorens-Bobadilla et al., 2015; Mizrak et al., 2020; Mizrak et al., 2019; Shi et al., 2018; Xie et al., 2020; Zamboni et al., 2020; Zywitza et al., 2018). Eight of these were chosen for further study because in-depth results of their analyses were available (Yan et al., 2022a). Papers and data were chosen that examined healthy brains in the absence of any treatment, genetic manipulation, or pathology.

Data available as supplementary spreadsheets from each study were first taken that examined enrichment in a selected cell type (NSCs, Nbs, TAPs) relative to all other cell types in the dataset. Each dataset was filtered to identify every occurrence in which an lncRNA was enriched with an adjusted p value < 0.05. The number of datasets in which a gene fit this criterion was summed to give cumulative occurrences (CO) for each category of comparison. The Ensembl database was accessed via R to annotate the gene biotype of each gene and to allow subsetting of data to isolate lncRNAs (Howe et al., 2021). Assessment of conservation was carried out using a combination of sequence-based searches (BLAST and discontinuous megaBLAST) and examination of conservation of synteny (Kent, 2002; Ma et al., 2002). This strategy was chosen to accommodate the fact that while a given lncRNA may be functionally conserved, the sequence conservation of lncRNAs is frequently poor. The papers used for the analysis are listed in Table 1.

Transcription factor binding motifs that were overrepresented within the promoters of murine lncRNAs were identified using the web tool PSCAN and the mouse genome reference database in a window from +450 upstream to −50 bp downstream of the annotated transcriptional start site for each gene (Zambelli et al., 2009). Transcription factors that were overrepresented within this sequence relative to a random control sequence with p < 0.05 were taken to examine GO. GO analysis was conducted for the cohort of transcription factors with binding sites in each promoter indivudlally via EnrichR in R with an adjusted p value cutoff of <0.05 (Kuleshov et al., 2016; Xie et al., 2021). The results of these separate GO analyses were then compared with find GO terms that were associated with every lncRNA promoter examined.

Sox2ot case study

ISH data and BrainSpan were accessed via the Allen Brain Atlas to examine the expression of Sox2ot, both anatomically and temporally (Sunkin et al., 2013). PSCAN was implemented via the webserver to identify transcription factors that are able to bind to the region −450 + 50 base pairs of the transcription start site of mouse *Sox2ot* and human *SOX2-OT*, using the mouse and human reference databases respectively (Zambelli et al., 2009). GO analysis for these transcription factors was performed using EnrichR via R (Kuleshov et al., 2016; Xie et al., 2021).

Miat case study

ISH data and BrainSpan were accessed via the Allen Brain Atlas to examine the expression of *Miat* both anatomically and temporally (Sunkin et al., 2013). PSCAN was implemented via webserver to identify transcription factors that are able to bind to the region −450 + 50 of the transcription start site of the mouse *Miat* and human *MIAT* (Zambelli et al., 2009). GO analysis for these transcription factors was performed using EnrichR via R (Kuleshov et al., 2016; Xie et al., 2021).

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.stemcr.2022.11.011.

AUTHOR CONTRIBUTIONS

J.B. and F.S. conceived and wrote the resource. J.B. carried out the analysis. B.S., F.A., K.V., and W.H. wrote the resource.

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CONFLICTS OF INTEREST

The authors declare no competing interests.

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